Level of renal function and serum erythropoietin levels independently predict anaemia post-renal transplantation

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Abstract

Background. Post-renal transplant anaemia is a potentially reversible cardiovascular risk factor. Graft function, immunosuppressive agents and inhibition of the renin-angiotensin system have been implicated in its aetiology. The evaluation of erythropoietin (EPO) levels may contribute to understanding the relative contributions of these factors.

Methods. Two-hundred and seven renal transplant recipients attending the Belfast City Hospital were studied. Clinical and laboratory data were extracted from the medical records and laboratory systems.

Results. Of the 207 patients (126 male), 47 (22.7%) were found to be anaemic (males, haemoglobin (Hb) < 12 g/dl, females Hb < 11 g/dl). The anaemic group had a significantly higher mean serum creatinine level (162.8 μmol/l vs 131.0 μmol/l, P < 0.001) and lower mean estimated glomerular filtration rate (eGFR) (41.5 ml/min vs 54.9 ml/min, P < 0.001) than the non-anaemic group. Individual immunosuppressive regimens were comparable between those with and those without anaemia. Angiotensin converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB) administration was not more prevalent in those with anaemia compared with those without (36.2 vs 38.8%, P = 0.88).

There was a significant inverse correlation between Hb levels and serum EPO levels (R = −0.29, P < 0.001), but not between EPO levels and eGFR (R = 0.02, P = 0.74). Higher EPO levels were predictive of anaemia, independent of eGFR in multivariate analysis.

Conclusion. Anaemia is common in post-renal transplant patients. The levels of renal function and serum EPO and not immunosuppressive regimens or ACE-I/ARB use, are strong and independent predictors of anaemia.

Keywords: anaemia; erythropoietin; renal transplantation

Introduction

Following transplantation, the majority of renal grafts function well, but with glomerular filtration rates (GFRs) consistently below gender-matched normal ranges for persons with a single kidney. Increasingly, renal transplant recipients are recognized as a specific category of persons with chronic kidney disease (CKD) and are at risk of a progressive fall in GFR with the associated complications of CKD.

The problem of anaemia following renal transplantation is increasingly recognized [1–4] but has not been well characterized in this group of CKD patients. Anaemia has been linked with significant cardiovascular morbidity and mortality in renal transplant recipients [5,6]. The prevalence of anaemia post-renal transplantation is estimated to be in the range of 20–40% [1–4]. Major aetiological factors implicated in its pathogenesis include presumed EPO deficiency and the adverse effects of prescribed medications on erythropoiesis. However, the relationship between anaemia, renal transplant function, immunosuppressive regimens and erythropoietin (EPO) levels has not been fully evaluated. We studied the prevalence of anaemia in stable renal transplant patients to determine how EPO levels relate to other aetiological risk factors implicated in its pathogenesis.

Subjects and methods

Clinical data

Over a 3-month period in 2004, we evaluated 223 consecutive recipients of deceased donor kidneys with a functioning renal graft attending follow-up at the Belfast City Hospital renal transplant clinic. Medical data were retrospectively collected.
by chart review and included gender, age, time from transplantation, details of immunosuppressive therapy and treatment with an angiotensin converting enzyme inhibitor (ACE-I) or an angiotensin-II type 1 receptor blocker (ARB).

**Laboratory data**

Laboratory data compiled included measurements of serum creatinine (\(\mu\)mol/l), estimated glomerular filtration rate (eGFR estimated with the validated equation from the Modification of Diet in Renal Disease study [7], which includes age, gender, race and serum creatinine), haemoglobin (Hb) (g/dl), mean cell volume (MCV) (fl) and serum albumin (g/l). Serum EPO levels (Quantikine® IVD® Erythropoietin enzyme-linked immunosorbent assay, R&D Systems Inc, Minneapolis, MN) were sampled on the morning of the patient’s routine transplant clinic visit.

Anaemia was defined as Hb < 12 g/dl for males and Hb < 11 g/dl for females consistent with the gender-specific K/DOQI (Kidney Disease Outcomes Quality Initiative) guidelines of 2001 [8].

**Statistical analysis**

Statistical analysis employed SPSS for Windows® (SPSS® Inc., Chicago, IL, USA) version 13.0. The Chi-squared test and Fisher’s exact test were performed for categorical variables and the independent t-test for continuous variables. Pearson’s correlation coefficient was calculated for correlation analysis. A multivariate linear regression model was used to consider associations between Hb levels and other variables and between EPO levels and other variables. A logistic regression model was employed to identify independent predictors of post-transplant anaemia.

Values of \(P < 0.05\) were considered statistically significant.

**Results**

Of the 223 consecutive patients recruited (\(>99\%\) Caucasian), nine patients within 3 months of renal transplant surgery and seven patients receiving exogenous recombinant human EPO were excluded from further analysis. Full data were available for the remaining 207 individuals of whom 126 (61\%) were male. The mean age was 49 years (range 14–82) and mean graft survival 97 months (range 4–412).

**Haemoglobin levels**

Anaemia was present in 47 patients (22.7\%). Of these, 26 males (20.6\% of males) had an Hb level < 12 g/dl and 21 females (25.9\% of females) an Hb < 11 g/dl (Figure 1). The characteristics of the anaemic and non-anaemic groups are detailed in Table 1. There were no significant differences in mean age of patients (46.3 years vs 49.2 years, \(P = 0.22\)) or graft survival (102.6 vs 95.2 months, \(P = 0.62\)) at the time of assessment between the anaemic and non-anaemic patients, respectively.

**Renal function**

The anaemic group had a significantly higher mean serum creatinine level (162.8 \(\mu\)mol/l vs 131.0 \(\mu\)mol/l, \(P < 0.001\)) and a lower mean eGFR (41.5 ml/min vs. 54.9 ml/min, \(P < 0.001\)) than the non-anaemic group. While the majority of patients in both groups had stage 3 CKD, a significantly higher proportion of those with anaemia had stage 4 disease compared with those without anaemia (27.7 vs 6.3\%, \(P < 0.001\)). Stage 1 and 2 disease was more prevalent in those without anaemia (12.7\% of the anaemic patients vs 35.0\% of the non-anaemic patients \(P = 0.002\)). Of the six patients with good renal function (stage 1 or 2) and anaemia none were prescribed an ACE-I/ARB, two were taking mycophenolate mofetil (MMF), two azathioprine (AZA) and two were on neither of these medications.

**Medication**

AZA (\(n = 37\)) or MMF (\(n = 101\)) was part of the immunosuppressive regimen in 63.8\% of anaemic patients vs 67.5\% of patients without anaemia (\(P = 0.73\)). Similarly, calcineurin inhibitors
(ciclosporin: \( n = 73 \), tacrolimus: \( n = 74 \)) were prescribed to 66% of anaemic patients compared with 72.5% of the non-anaemic renal transplant recipients (\( P = 0.45 \)). Only 15 patients were prescribed sirolimus, 4 of the anaemic and 11 of the non-anaemic patients (\( P = 0.96 \)). Seventy-nine patients were receiving an ACE-I (63) or an ARB (16), 36.2% of the anaemic and 38.8% of the non-anaemic groups (\( P = 0.88 \)). There had been no recent alterations to the individual immunosuppressant regimens prior to data collection.

**Erythropoietin levels**

The mean EPO level was higher in the anaemic patients than the non-anaemic patients although statistical significance was not reached (19.8 vs 12.4 U/l, \( P = 0.08 \)). When considering Hb levels in the whole cohort, there was a significant negative correlation between haemoglobin levels and EPO levels (Pearson’s correlation coefficient, \( R = -0.29, P < 0.001 \)), i.e. those with lower haemoglobin levels had higher EPO levels and vice versa. Interestingly, despite the significant correlation between Hb levels and eGFR (\( R = 0.37, P < 0.001 \)) there was not a significant correlation between EPO levels and eGFR (\( R = 0.02, P = 0.74 \)) (Table 2). This implies that graft function is not the principal determinant of serum EPO levels in these renal transplant recipients. Recipient age and gender were not significant variables in determining serum EPO levels.

Considering only the patients who were anaemic, there was however a significant correlation between EPO levels and eGFR (\( R = 0.34, P = 0.02 \)), as well as between EPO levels and Hb (\( R = -0.35, P = 0.02 \)) (Table 2). These factors remained significant in multivariate regression analysis. The Hb level within the anaemic cohort was significantly associated with eGFR, serum EPO levels and gender in multivariate regression analysis. The prescribed medications and age of the patient were not significant variables.

**Other variables**

Iron studies are not routinely requested in our renal transplant review clinic and were available for a limited number of patients (\( n = 12 \)) precluding meaningful analysis. However, the MCV was available for all recipients and was outside the normal laboratory range of 76–100 fl in only one of the 207 patients (MCV 72 fl, Hb 10.5 g/dl). The mean MCV was comparable between those who were and who were not anaemic (89.4 and 91.0 fl, \( P = 0.10 \)). Albumin can be considered as a surrogate marker for nutritional status and as a negative inflammatory marker.
The overall mean serum albumin level in this population was 41.1 g/l (normal range 35–50 g/l). The difference in mean albumin concentration between the anaemic and non-anaemic groups was 1.4 g/l, which was statistically significant (40.0 and 41.4, \( P = 0.03 \)), but not clinically significant as both mean values were maintained well within the normal range. Five recipients had below normal albumin levels (range 30–33) with an average Hb of 10.6 g/dl.

Early post-transplant anaemia may reflect pathophysiological mechanisms that are distinct from those contributing to low Hb levels in longstanding recipients. Some of these variables, such as peri-operative blood loss and poor initial graft function, are minimized by the exclusion of recipients within 3 months of transplantation in our study. Comparison of the 27 patients in the first post-transplant year with the remaining 180 patients in our study demonstrated no significant difference in mean Hb levels, proportion of anaemic patients, renal function or serum EPO levels. The only factors that differed significantly between the two groups were the immunosuppressive regimens and the prescription of renin-angiotensin system blocking medication. The absence of demonstrable differences between the recipients within a year of engraftment and the others may reflect the small numbers in the first group: only 4 out of 47 (8.5%) of the patients with anaemia were recent transplant recipients.

**Predictors of anaemia**

In multivariate regression analysis, independent predictors of lower Hb levels were lower eGFR, higher EPO levels, younger age and female gender. However, female gender was no longer significant when a logistic regression model was used to determine the predictors of anaemia. The eGFR \( (P < 0.001) \), serum EPO level \( (P < 0.001) \) and recipient age \( (P = 0.006) \) were the only independent predictors of anaemia in our post-renal transplant population.

**Discussion**

Anaemia was common affecting 22.7% of this cohort of stable post-renal transplant patients. This is consistent with the reported prevalence of 20–40% in other studies of post-renal transplant anaemia [1–4,9–13]. The Transplant and European Survey on Anaemia Management (TRESAM) [3] included 4263 renal transplant recipients from 72 centres across Europe. This cohort was 60% male with a mean age of 45.5 years, similar to our patient group. Anaemia was reported in 38.6% of these patients and was equally distributed between genders. However, their threshold for anaemia (Hb \( \leq 13 \) g/dl for males and Hb \( \leq 12 \) g/dl for female) was less stringent than ours (Hb \( < 12 \) g/dl for males and Hb \( < 11 \) g/dl for females). The definition of anaemia has varied across many of the studies making valid comparisons difficult and contributing to the variation in results [2,9,10]. Indeed the K/DOQI guidelines recently published now have a single definition of anaemia (Hb \( < 11 \) g/dl) across both genders [14]. In our study, in keeping with the TRESAM data, the prevalence of anaemia did not differ by gender. Of interest, the literature is inconsistent in this area with some studies reporting a preponderance of anaemia in males [2,9] others in females [12].

We noted a strong correlation between level of renal function and anaemia, with the anaemic group having a significantly lower eGFR and higher serum creatinine levels. This is unsurprising and is consistent with the other studies that have reported on post-renal transplant anaemia [2–4, 10–12].

Contrary to several previous investigations, we found no relationship between anaemia and immunosuppressive regimens. TRESAM [3,4] reported that the use of MMF or AZA was associated with anaemia (odds ratio, OR: 1.24, \( P < 0.05 \)). MMF was also implicated by Yorgin and co-workers [2] and Winkelmayer and colleagues [11]. The latter group also reported tacrolimus was independently associated with anaemia. More recently, anaemia was reported to be more prevalent in patients prescribed MMF or sirolimus, although the authors felt that this may reflect poorer graft function in these recipients [13], illustrating the difficulties in establishing a causal relationship between specific immunosuppressive regimens and anaemia. We found no evidence that specific immunosuppressive regimens contributed significantly to post-renal transplant anaemia using univariate or multivariate analysis.

Our patients who were prescribed an ACE-I or ARB medication were at no added risk of anaemia. The published literature in this area is again inclusive. Associative logistic regression used in the TRESAM study [3,4] identified the use of ACE-I or ARB medications to be associated with a higher OR for being anaemic (OR = 1.55, \( P < 0.001 \)). Winkelmayer and colleagues [11] concurred with this finding. As these studies are cross-sectional and retrospective, this finding may not be causal but represents the increased use of renin-angiotensin system blockade medication in patients with progressive CKD associated with hypertension and proteinuria. Our results, however, are consistent with those of Fernandez Fresno and colleagues [15] whose prospective data found no correlation between anaemia and ACE-I/ARB use in 397 de novo and 2102 maintenance renal transplant recipients.

The role of endogenous EPO production in the setting of post-transplant anaemia has not been fully elucidated and few studies have included serum EPO levels in their analyses [16–18]. Theoretically, the hypothesis that post-renal transplant anaemia is the result of relative EPO deficiency secondary to poor graft function is plausible, but the response to EPO after transplantation appears to differ mechanistically from that observed with chronic dialysis therapy. In the latter, large and sustained doses of exogenous EPO...
therapy are generally required; after renal transplantation a transient rise in EPO levels are reported with persistent erythropoiesis despite a return to normal EPO levels when the serum haematocrit reaches 0.32 [16]. It is postulated that additional factors enhance the sensitivity to endogenous EPO and facilitate the maintenance of erythropoiesis. A smaller study concluded that relative or absolute EPO deficiency can persist in post-renal transplant recipients despite restoration of normal renal function [17]. Despite our findings that the eGFR is the strongest predictor of anaemia in our population, there was no significant correlation between EPO levels and eGFR in our patients. Only in those patients who were anaemic was a significant relationship identified. Consistent with these findings, in multivariate regression analysis serum EPO levels were negatively predictive of Hb levels and also of anaemia independently of renal function. This implies that non-renal factors, distinct from immunosuppressive regimens and ACE-I/ARB prescription, are important in post-renal transplant anaemia.

Our study has limitations intrinsic to retrospective and cross-sectional data and the sample size is smaller than in some other reports. It is however, a less heterogeneous population than other studies, as virtually all (>99%) were Caucasian, all were recipients of deceased donor kidneys and none were prescribed exogenous EPO therapy. Other studies have included patients receiving erythropoiesis stimulating agents, classifying such patients as anaemic irrespective of their haemoglobin level, with potential for the confounding of results [11,13]. Information on other variables such as iron stores, parathormone levels and C-reactive protein would have contributed usefully to our study, but was not available at the time of analysis.

Post-renal transplant anaemia is common and has a multifactorial aetiology. Serum EPO levels were negatively predictive of anaemia in our population, independent of the level of renal function. Our findings imply that non-renal factors, distinct from individual immunosuppressive regimens and renin-angiotensin system blockade, are important in post-renal transplant anaemia. We suggest that future studies, using a uniform definition of gender-specific anaemia, should focus on prospective data collections in cohorts from the time of renal transplantation and include iron indices and EPO levels given the findings in this study.

Conflict of interest statement. None declared.

References

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