Posttransplantation anemia in kidney transplant recipients

A retrospective cohort study

Anat Gafter-Gvili, MD[a,b,*], Irit Ayalon-Dangur, MD[b,c], Lisa Cooper, MD[b,d], Tzippy Shochat[e], Ruth Rahamimov, MD[b,f], Uzi Gafter, MD, PHD[b,f], Eytan Mor, MD[b,g], Alon Grossman, MD[b,c]

Abstract

We sought to assess the frequency and predictors of early and late posttransplantation anemia (PTA). In addition, we aimed to assess the outcomes of patients with anemia and to assess the impact of anemia on mortality, graft function, and graft failure.

Patients who underwent kidney transplantation in a single center during a 4-year period were included. Predictors associated with the development of anemia at 6 months (early PTA) or 2 years (late PTA) were evaluated in a univariate and multivariate analyses. The effects of anemia and other variables on mortality and graft function were assessed.

A total of 266 kidney transplant recipients were included. The prevalence of PTA at 6 months (early PTA) was 51.3% and at 2 years (late PTA) was 36.6%. Female sex was significantly associated with early PTA. Patients with early PTA proceeded to late PTA. Patients with both early and late PTA had a higher mortality rate at 4 years compared to patients without anemia. On multivariable analysis, lower Hb at 2 years posttransplantation (hazard ratio [HR] 0.716, 95% confidence intervals [CI] 0.541–0.948, for every increment of 1 g/dL) was significantly associated with mortality. Patients with late PTA suffered a decline in eGFR compared to patients without anemia ($P = .026$). Furthermore, a lower Hb at 2 years posttransplantation was also associated with graft failure (HR 0.775, 95% CI 0.619–0.969, for every increment of 1 g/dL).

Post-transplantation anemia is significantly associated with late mortality, with a decline in graft function and with an increased incidence of graft failure.

Abbreviations: ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blockers, CKD = chronic kidney disease, CKD-EPI = chronic kidney disease epidemiology collaboration, DM = diabetes mellitus, eGFR = estimated glomerular filtration rate, Hb = hemoglobin, MCV = mean corpuscular volume, NODAT = new onset diabetes after transplantation, PTA = posttransplantation anemia, RBCs = red blood cells, RDW = red cell distribution width.

Keywords: anemia, kidney transplantation, posttransplantation anemia, PTA

1. Introduction

Anemia frequently complicates chronic kidney disease (CKD) and has been shown to be associated with increased mortality in dialysis patients.[1] Anemia may potentially be corrected by kidney transplantation, yet posttransplantation anemia (PTA) is estimated to be in the range of 20% to 40% of patients.[2–6]

PTA has not been uniformly defined in terms of timing after transplantation and degree of anemia. Some authors suggest distinguishing between early PTA (up to 6 months) and late PTA (after 6 months and up to 5 years).[5,7]

The most common reason for early PTA is iron deficiency.[8] Iron deficiency may be caused by depletion of iron stores before transplantation and perioperative blood loss.[8,9] In addition, slowly increasing levels of the newly graft-produced-erythropoietin may contribute to the anemia.[10,11] The occurrence of late PTA has been associated with impaired graft function and the development of renal insufficiency.[2]

Several studies have shown that PTA may be associated with increased mortality[12–14] and decreased graft survival,[15–17] and of de-novo congestive heart failure.[18]

We sought to assess the frequency of early and late PTA, and to find predictors, in order to help define subgroups at risk for the development of anemia. In addition, we aimed to assess the impact of anemia on clinical outcomes—mortality, graft function, and graft failure.

2. Materials and methods

2.1. Study design and patients

This is a single-center retrospective cohort study using the Rabin Medical Center (RMC) transplantation department registry. This registry prospectively collects data regarding the recipients'

[a] Department of Medicine A, Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel, [b] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [c] Department of Medicine E, Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel, [d] Department of Geriatrics, Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel, [e] Bio-Statistical Unit, Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel, [f] Department of Nephrology and Hypertension, Rabin Medical Center, Petah Tikva, Israel, [g] Department of Transplantation, Rabin Medical Center, Beilinson Campus, Israel.

* Correspondence: Anat Gafter-Gvili, Rabin Medical Center, Petah-Tikva, Israel (e-mail: anatga2@clalit.org.il).

Gafter-Gvili et al. Medicine (2017) 96:32

Medicine

characteristics and outcomes. Adult patients (age >18 years) who underwent kidney transplantation in our center during a 4-year period between 1 January 2008 and 31 December 2011 were included. Due to the reported association between diabetes mellitus (DM) and anemia,[19,20] only patients without DM before the transplantation were included in the analysis. The study was approved by the RMC institutional review board.

2.2. Definitions

Patients were classified as having PTA based on the Hb levels at 6 months (defined as early PTA) and at 2 years (defined as late PTA). Anemia was defined according to World Health Organization (WHO) and American Society of Transplantation guidelines as hemoglobin levels <13 mg/dL in men and <12 mg/dL in women.[21,22]

2.3. Data collection

Data relevant to the diagnosis of anemia and anemia workup were evaluated at 3 time points: before transplantation (maximum 6 months prior), 6 months, and 2 years following transplantation. Anemia workup included mean corpuscular volume (MCV), red cell distribution width (RDW), iron, transferrin, ferritin, percentage of hypochromic red blood cells (RBCs), vitamin B_{12}, folic acid, thyroid-stimulating hormone (TSH), erythropoietin level, reticulocyte count, haptoglobin, lactate dehydrogenase bilirubin, and vitamin D.

Pretransplantation characteristics were collected. Post-transplantation laboratory variables included estimated glomerular filtration rate (eGFR) values, calculated according to the chronic kidney disease epidemiology collaboration (CKD-EPI) 2009 equation,[23] and the number of patients with CKD, defined as eGFR <60 mL/min, at 6 months and 2 years after transplantation (the time of anemia workup). The occurrence of new onset diabetes after transplantation (NODAT) is defined according to the American Diabetes Association criteria for the diagnosis of DM.[24]

The use of maintenance immunosuppressive therapy, lipid-lowering medications, angiotensin-converting enzyme (ACE) inhibitors, or angiotensin receptor blockers (ARB), antiplatelets, and use of trimethoprim–sulfamethoxazole administered as pneumocystis jiroveci prophylaxis were documented.

2.4. Outcomes

The primary outcome of the study was all-cause mortality at the end of follow-up. We compared all-cause mortality between those who developed PTA and those who did not in order to evaluate the effect of anemia on mortality. Secondary outcomes included graft failure (defined as re-establishment of long-term dialysis therapy, the need for retransplantation or death), change in kidney function (defined as the difference in eGFR between eGFR at 2 years and at 6 months), and major adverse cardiovascular events (including myocardial infarction, revascularization, stroke, and acute coronary syndrome).

2.5. Statistical analysis

The statistical analysis was generated using SAS Software, Version 9.4. Continuous variables were presented by mean± standard deviation, and categorical variables were presented by

(N, %). T-Test was used to compare the value of continuous variables between study groups. The χ^2 (for >2 groups) or Fisher exact (for 2 groups) tests were used to compare the value of categorical variables between study groups. Two-sided P values <.05 were considered statistically significant. Characteristics of patients who developed early and late PTA were compared in both univariate and multivariate analyses in order to find predictors for PTA. Time-to-event outcomes (mortality and graft failure) were compared using Kaplan–Meier survival analysis using the log-rank test. We evaluated the effect of the above variables on overall survival and graft failure in a univariate analysis. Variables found to be significantly associated with mortality (P <.05) were entered into a Cox regression multivariate analysis. Hazard ratios (HRs) with 95% confidence intervals (CI) were calculated.

3. Results

3.1. Study population, anemia prevalence, and follow-up

During the study period, 398 individuals underwent kidney transplantation at RMC. Of these, 132 were excluded due to pretransplantation DM. A total of 266 kidney transplant recipients were included in the study, of which 261 could be evaluated for anemia at 6 months and 254 could be evaluated at 2 years. Baseline characteristics of the cohort, according to development of PTA at 6 months and at 2 years are shown in Tables 1 and 2. The prevalence of anemia pretransplantation was 71% (184/259) with a mean pretransplantation Hb of 11.6±1.6 g/dL. The prevalence of PTA at 6 months (early PTA) was 51.3% (134/261) with a mean Hb of 12.54±2.05 g/dL, and the prevalence of PTA at 2 years (late PTA) was 36.6% (93/254) with a mean Hb of 13.18±2.47 g/dL. Mean follow-up time for the cohort was 5.46 ±1.21 years.

3.2. Risk factors for early and late PTA

Baseline characteristics pretransplantation associated with early PTA on univariate analysis included young age, low weight, and low cholesterol level. More patients with anemia had a history of a cerebrovascular accident. Hematological parameters included a higher percentage of hypochromic RBCs pretransplantation and at 6 months posttransplantation, as well as a higher RDW at 6 months posttransplantation. Other factors posttransplantation associated with early PTA included a lower eGFR, the presence of CKD, a lower vitamin D level, and proteinuria (Table 1). These factors, in addition to gender, were entered into a multivariate analysis, and those that remained significantly associated with early PTA were female sex (HR 3.13, 95% CI 1.180–8.284), lower eGFR (HR 0.97, 95% CI 0.95–0.99, for every increment of 1 mL/min/1.73 m²), and hypochromic RBCs (HR 1.053, 95% CI 1.001–1.1107).

Baseline characteristics pretransplantation associated with late PTA on univariate analysis included female sex, low weight, a lower triglycerides level, and a lower percentage of a living donor as the transplantation source (Table 2). Hematological parameters included a higher RDW and a higher B_{12} level pretransplantation and at 6 months posttransplantation, a lower Hb level 6 months posttransplantation and a lower iron level 2 years posttransplantation. Other factors posttransplantation associated with late PTA included lower eGFR and the presence of CKD, at 6 months and 2 years, proteinuria at 6 months, and the use of cyclosporine (Table 2).

Table 1

Clinical characteristics of patients with anemia at 6 months (early PTA).

Characteristic	Patients with anemia (n = 134)	Patients without anemia (n = 127)	*P* value
Baseline characteristics pretransplant			
Age at transplantation, years	44.02 ± 16.56	47.78 ± 13.28	.044
Female, number, (%)	61 (45.5)	44 (34.9)	.082
Weight, kg	69.03 ± 16.82	74.03 ± 16.88	.024
Systolic blood pressure, mm Hg	141.37 ± 27.46	141.44 ± 22.51	.992
Diastolic blood pressure, mm Hg	85.92 ± 15.56	86.64 ± 12.8	.698
HTN	90/118 (76.3)	97/121 (80.2)	.466
CVA	12/118 (10.2)	3/120 (2.5)	.015
IHD	10/118 (8.5)	16/121 (13.2)	.239
CHF	5/118 (4.2)	9/121 (7.4)	.292
Smoking, number (%)	39/134 (29.1)	40/127 (31.5)	.989
Dialysis, number (%)	104/134 (77.6)	104/127 (81.9)	.391
Living donor, number (%)	76/134 (56.7)	80/127 (63)	.301
Baseline laboratory parameters pretransplant			
Cholesterol, mg/dL	163.7 (50.27)	175.93 (41.87)	.033
LDL, mg/dL	80.82 (36.08)	89.12 (30.27)	.054
Triglycerides, mg/dL	171.47 (97.87)	189.02 (122.6)	.206
Proteinuria, number (%)	130/134 (97)	125/127 (98)	.054
Baseline hematological parameters pretransplant			
Anemia, n (%)	96 (72.73)	88 (69.8)	.6
Hemoglobin, g/dL (SD)	11.46 (1.48)	11.77 (1.75)	.114
Mean corpuscular volume, fl (SD)	90.85 (6.74)	92.31 (6.51)	.079
Red cell distribution width,% (SD)	14.88 (1.59)	14.71 (1.58)	.382
Ferritin, ng/mL (SD)	454.29 (475.08)	495.77 (421.56)	.564
Iron, ng/mL (SD)	74.17 (40)	71.84 (31.57)	.665
Transferrin, ng/mL (SD)	185.36 (46.01)	181.14 (45.71)	.551
hypochromic RBCs, % (SD)	5.54 (6.48)	3.86 (4.36)	.025
Vitamin B_{12}, pmol/L (SD)	481.77 (275.41)	439.7 (253.55)	.303
Folic acid, nmol/L (SD)	23.39 (19.19)	20.38 (16.98)	.300
TSH, mIU/L (SD)	2.58 (1.87)	2.19 (1.37)	.137
Lactate dehydrogenase, U/L (SD)	395.14 (42.7)	439.33 (276.64)	.714
Haptoglobin, mg/dL (SD)	130.96 (122.28)	123.10 (53.7)	.896
Vitamin D, nmol/L	19.99 (9.48)	20.11 (9.9)	.966
Hematological and other laboratory parameters at 6 months posttransplant			
Hemoglobin, g/dL (SD)	11.11 (1.21)	14.05 (1.31)	<.001
Mean corpuscular volume, fl (SD)	88.55 (6.54)	89.34 (5.52)	.294
Red cell distribution width,% (SD)	14.37 (1.44)	13.9 (1.00)	.002
Ferritin, ng/mL (SD)	352.72 (377.69)	480.3 (550.65)	.06
Iron, ng/mL (SD)	73.89 (33.28)	78.77 (32.06)	.278
Transferrin, ng/mL (SD)	216.35 (55.61)	211.48 (51.63)	.520
Hypochromic RBCs, % (SD)	7.19 (10.28)	4.93 (6.38)	.048
Vitamin B_{12}, pmol/L (SD)	427.99 (230.27)	385.40 (224.52)	.179
Folic acid, nmol/L (SD)	22.26 (17.95)	20.94 (15.82)	.585
TSH, mIU/L (SD)	2.23 (1.56)	2.05 (1.25)	.378
EPO mIU/mL (SD)	34.72 (92.49)	12.92 (7.7)	.363
Lactate dehydrogenase, U/L (SD)	572.92 (206.27)	591.60 (487.09)	.790
Haptoglobin, mg/dL (SD)	120.19 (64.58)	133.20 (61.77)	.704
Vitamin D, nmol/L	16.13 (6.57)	19.10 (9.36)	.028
eGFR, mL/min/1.73 m^2 (SD)	54.04 (23.09)	60.87 (20.06)	.013
CKD, n (%)	80 (62.5)	59 (46.83)	.016
NODAT, n (%)	41 (30.6)	29 (22.83)	.157
Proteinuria, n (%)	90 (67)	74 (58)	.006
Immunosuppressive drugs, other drugs, and prophylactic antibiotics, no. (%)			
Tacrolimus	117 (87)	118 (92.9)	.131
Cyclosporine	13 (9.7)	6 (4.72)	.122
Mycophenolate mofetil/ mycophenolate sodium	128 (95.55)	124 (97.64)	.349
Prednisone	133 (99.25)	127 (100)	.329
ACE-inhibitor/ARB	32 (27.12)	27 (22.31)	.454
Beta blockers	51 (43.22)	55 (45.45)	.795
Diuretics	22 (18.64)	20 (16.53)	.735
Statins	36 (30.51)	31 (25.62)	.472
Aspirin	24 (20.34)	29 (23.97)	.536
Clopidogrel	4 (3.4)	1 (0.83)	.209
Trimethoprim–sulfamethoxazole	91 (67.9)	103 (81.1)	.016

All continuous data are presented as mean ± SD.

Dichotomous data as number of patients and percentages within parentheses.

ACE-inhibitor = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, CHF = chronic heart failure, CKD = chronic kidney disease, CVA = cerebrovascular accident, EPO = erythropoietin, HTN = hypertension, IHD = ischemic heart disease, LDL = low-density lipoprotein, n = number, RBC = red blood cell, NODAT = new onset diabetes after transplant, PTA = posttransplantation anemia, SD = standard deviation, TSH = thyroid-stimulating hormone.

Gafter-Gvili et al. Medicine (2017) 96:32

Medicine

Table 2

Clinical characteristics of patients with anemia at 2 years (late PTA).

Characteristic	Patients with anemia (n = 93)	Patients without anemia (n = 161)	*P* value
Baseline characteristics pretransplant			
Age at transplantation	46.26 ± 17.22	45.20 ± 14.11	.617
Female, number (%)	50 (53)	50 (34.9)	<.001
Weight, kg	65.44 ± 14.97	74.27 ± 17.26	<.001
Systolic blood pressure, mm Hg	144.83 ± 28.53	139.63 ± 23.19	.160
Diastolic blood pressure, mm Hg	86.94 ± 15.86	85.79 ± 13.26	.557
HTN	62/82 (75.6)	123/156 (78.9)	.568
CVA	7/82 (8.5)	8/155 (5.2)	.310
IHD	9/82 (11)	17/156 (11)	.985
CHF	4/82 (4.9)	10/156 (6.4)	.633
Smoking, number (%)	26/93 (28)	53/161 (32.9)	.635
Dialysis, number (%)	78/93 (83.9)	125/161 (77.6)	.232
Living donor, number (%)	43/93 (46.2)	109/161 (67.7)	<.001
Baseline laboratory parameters pretransplant			
Cholesterol, mg/dL	168.5 (51.02)	170.36 (44.5)	.759
LDL, mg/dL	85.33 (37.62)	84.7 (31.05)	.895
Triglycerides, mg/dL	159.48 (89.18)	190.02 (120.8)	.023
Proteinuria, number (%)	92/93 (99)	134/161 (83.2)	.671
Baseline hematological parameters pretransplant			
Anemia, n (%)	68 (73.91)	109 (69.4)	.451
Hemoglobin g/dL (SD)	11.38 (1.58)	11.77 (1.62)	.061
Mean corpuscular volume, fl (SD)	91.55 (6.7)	91.48 (6.63)	.933
Red cell distribution width,% (SD)	14.88 (1.59)	14.71 (1.58)	.035
Ferritin, ng/mL (SD)	514.57 (513.04)	427.77 (394.04)	.267
Iron, ng/mL (SD)	70.58 (37.62)	72.85 (32.83)	.675
Transferrin, ng/mL (SD)	179.66 (48.56)	186.86 (43.35)	.322
hypochromic RBCs, % (SD)	5.54 (6.48)	3.86 (4.36)	.057
Vitamin B$_{12}$, pmol/L (SD)	481.77 (275.41)	439.7 (253.55)	.012
Folic acid, nmol/L (SD)	23.39 (19.19)	20.38 (16.98)	.293
TSH, mIU/L (SD)	2.58 (1.87)	2.19 (1.37)	.071
Lactate dehydrogenase, U/L (SD)	395.14 (42.7)	439.33 (276.64)	.803
Haptoglobin, mg/dL (SD)	130.96 (122.28)	123.10 (53.7)	.858
Vitamin D, nmol/L	19.99 (9.48)	20.11 (9.9)	.741
Hematological and other laboratory parameters at 6 months posttransplant			
Hemoglobin g/dL (SD)	11.54 (1.57)	13.24 (1.77)	<.001
Mean corpuscular volume, fl (SD)	89.72 (5.86)	88.22 (6.29)	.063
Red cell distribution width, % (SD)	14.35 (1.36)	13.96 (1.12)	.018
Ferritin, ng/mL (SD)	514.57 (513.04)	427.77 (394.04)	.371
Iron, ng/mL (SD)	70.58 (37.62)	72.85 (32.83)	.216
Transferrin, ng/mL (SD)	179.66 (48.56)	186.86 (43.45)	.149
Hypochromic RBCs, % (SD)	5.84 (6.79)	4.16 (4.81)	.179
Vitamin B$_{12}$, pmol/L (SD)	540.32 (315.09)	423.37 (227.72)	.015
Folic acid, nmol/L (SD)	23.82 (19.41)	20.70 (17.05)	.293
TSH, mIU/L (SD)	2.72 (2.14)	2.17 (1.25)	.320
EPO, mIU/mL (SD)	23.38 (12.7)	7.83 (3.47)	.320
Lactate dehydrogenase, U/L (SD)	401.00 (106.4)	428.00 (238.38)	.196
Haptoglobin, mg/dL (SD)	123.10 (91.39)	133.58 (114.83)	.361
Vitamin D, nmol/L	20.75 (11.39)	19.74 (9.08)	.812
eGFR, mL/min/1.73 m^2 (SD)	52.01 (24.05)	61.70 (20.02)	.001
CKD, n (%)	62 (67.39)	76 (47.2)	.002
NODAT, number (%)	24 (25.8)	45 (27.9)	.711
Proteinuria, number (%)	66 (71)	96 (60)	.002
Hematological and other laboratory parameters at 2 year posttransplant			
Ferritin, ng/mL (SD)	351.72 (422.09)	370.01 (582.28)	.817
Iron, ng/mL (SD)	68.56 (39.23)	79.92 (32.11)	.032
Transferrin, ng/mL (SD)	212.86 (51.74)	224.82 (51.84)	.132
Vitamin B$_{12}$, pmol/L (SD)	374.73 (215.02)	363.23 (191.59)	.713
Folic acid, nmol/L (SD)	18.23 (14.1)	18.08 (11.97)	.939
eGFR, mL/min/1.73 m^2 (SD)	50.59 (27.85)	64.69 (23.3)	<.001
CKD, n (%)	59 (64.13)	65 (40.63)	<.001
Immunosuppressive Drugs and prophylactic antibiotics, no. (%)			
Tacrolimus	76 (81.7)	152 (94.4)	.001
Cyclosporine	13 (13.9)	6 (3.7)	.003
Mycophenolate mofetil/mycophenolate sodium	88 (94.6)	156 (96.9)	.370
Prednisone	92 (98.9)	161 (100)	.187
ACE-inhibitor/ARB	18 (21.95)	37 (23.72)	.634
Beta blockers	32 (39.02)	73 (46.79)	.274
Diuretics	14 (17.07)	28 (17.95)	1.000
Statins	25 (30.49)	42 (26.92)	.649
Aspirin	18 (21.95)	37 (23.72)	.872
Clopidogrel	3 (3.66)	2 (1.28)	.343
Trimethoprim–sulfamethoxazole	64 (68.8)	130 (80.8)	.031

All data are presented as mean ± SD.

Anemia defined as hemoglobin levels <13 mg/dL in males or <12 mg/dL in females.

ACE-inhibitor = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, CHF = chronic heart failure, CKD = chronic kidney disease, CVA = cerebrovascular accident, eGFR = estimated glomerular filtration rate, EPO = erythropoietin, HTN = hypertension, IHD = Ischemic heart disease, LDL = low-density lipoprotein, n = number, NODAT = new onset diabetes after transplant, PTA = posttransplantation anemia, RBC = red blood cell, RDW = red cell distribution width, SD = standard deviation, TSH = thyroid-stimulating hormone.

Table 3

Outcomes of patients with anemia.

Outcome	Patients with anemia	Patients without anemia	*P* value
Early PTA			
Mortality (4 years)	12/134 (8.96%)	3/127 (2.36%)	.022
Graft failure (4 years)	25/134 (18.66%)	8/127 (6.3%)	.003
Cardiovascular outcome (4 years)	11/134 (8.2%)	11/127 (8.66%)	.89
Late PTA			
Mortality (4 years)	8/93 (8. 6%)	2/161 (1.24%)	.004
Graft failure (4 years)	22/93 (23.66%)	5/161 (3.11%)	<.001
Cardiovascular outcome (4 years)	11/93 (11.8%)	10/161 (6.21%)	.12
Delta eGFR mL/min/1.73 m² (SD)	−2.26 (19.29)	3 (14.74)	.026

Delta eGFR is the difference in eGFR between 2 years and 6 months, presented as mean ± SD.
eGFR = estimated glomerular filtration rate, PTA = posttransplantation anemia, SD = standard deviation.

On multivariable analysis, the hematological factors which remained significantly associated with late PTA included: a lower iron level at 2 years posttransplantation (HR 0.986, 95% CI 0.974–0.998, for every increment of 1 mg/dL), and a lower Hb level at 6 months posttransplantation (HR 0.601, 95% CI 0.453–0.799, for every increment of 1 g/dL). Other factors included a low body weight pretransplantation (HR 0.957, 95% CI 0.925–0.989, for every increment of 1 kg), living donor as the transplantation source (HR 0.343, 95% CI 0.142–0.831), and a low triglycerides level pretransplantation (HR 0.993, 95% CI 0.986–0.998, for every increment of 1 mg/dL).

3.3. Outcomes of patients with anemia

Patients with early PTA had a higher mortality rate at 4 years (8.96% vs 2.36%, *P* = .022) and a higher percentage of graft failure (18.66% vs 6.3%, *P* = .003) (Table 3).

Patients with late PTA also had a higher mortality rate at 4 years (8.6% vs 1.24%, *P* = .004) (Fig. 1) and a higher percentage of graft failure (23.66% vs 3.11%, *P* < .001).

In patients with late PTA, there was a decline in kidney function. The eGFR declined with time, with a difference between eGFR at 2 years and at 6 months of 2.26 mL/min/1.73 m² (SD 19.29) in patients with anemia, in contrast to a rise of 3 mL/min/1.73 m² (SD 14.74) in patients without anemia (Table 3). Hence, there was a significant difference of 5.3 mL/min/1.73 m² in eGFR between patients with and without anemia (*P* = .026).
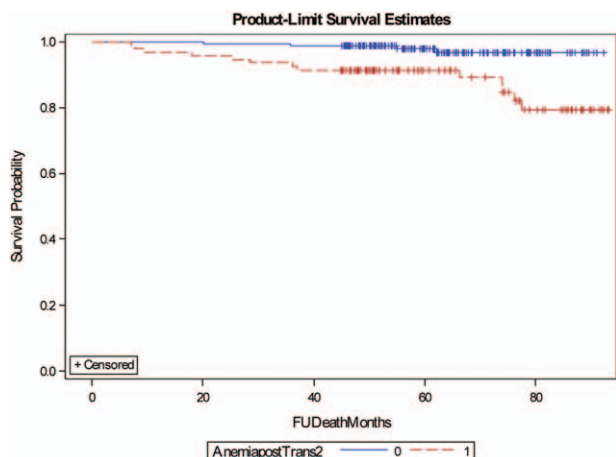
Figure 1. Overall survival of patients according to the presence of anemia at 2 years. Blue curve—patients without anemia, red curve—patients with anemia.

There was no difference in the frequency of cardiovascular events between patients with early and late PTA and those without them (Table 3).

3.4. Risk factors for mortality

Fifteen patients died during follow-up (5.6% mortality rate). Risk factors for 4-year mortality on univariate analysis included: older age at transplantation, a lower Hb level at 6 months and 2 years, a higher RDW and a lower iron level at 6 months, a lower folic acid level at 6 months posttransplantation, and a lower iron level at 2 years posttransplantation. In addition, living donor as the transplantation source was associated with a less mortality. All these variables in addition to gender were included in the multivariable model. On multivariable analysis, the factors that remained significantly associated with mortality were older age (HR 1.123, 95% CI 1.040–1.213, for every increment of 1 year) and a lower Hb at 2 years posttransplantation (HR 0.716, 95% CI 0.541–0.948, for every increment of 1 g/dL) (Table 4).

3.5. Risk factors for graft failure

Risk factors for graft failure at 4 years on univariate analysis included: older age at transplantation, the following parameters at 6 months posttransplantation: a lower Hb level, lower iron level, a higher RDW and a higher TSH, and a lower Hb level at 2 years posttransplantation. In addition, the presence of CKD and lower eGFR at 6 months and 2 years were associated with graft failure.

On multivariable analysis, the following factors were found to be significantly associated with graft failure: male sex (HR 0.453, 95% CI 0.212–0.969), lower Hb at 2 years posttransplantation

Table 4

Multivariable analysis for mortality.

Variable	HR	95% CI	*P*
Gender (female)	1.140	0.329–3.946	.836
Age (older), (per 1-year increment)	1.123	1.040–1.213	.003
Hb at 6 months post (lower), per 1 g/dL increment	0.744	0.512–1.081	.121
Hb at 2 years post (lower), per 1 g/dL increment	0.716	0.541–0.948	.019
RDW at 6 months post (higher)	0.830	0.485–1.420	.496
Transplant source—living donor	2.703	0.662–11.046	.166
Iron at 6 months post (lower)	0.980	0.956–1.004	.102

CI = confidence interval, Hb = hemoglobin, HR = hazard ratio, RDW = red cell distribution width

Gafter-Gvili et al. Medicine (2017) 96:32

Medicine

(HR 0.775, 95% CI 0.619–0.969, for every increment of 1 g/dL), lower iron level at 6 months posttransplantation (HR 0.986, 95% CI 0.974–0.998, for every increment of 1 mg/dL), lower eGFR at 2 years (HR 0.927, 95% CI 0.900–0.956), and an elevated TSH level (HR 1.265, 95% CI 1.134–1.411).

4. Discussion

In this single-center retrospective cohort study of 266 renal transplant recipients, we attempted to define the prevalence of early and late PTA, to find predictors for anemia and to evaluate their effect on patient and graft survival.

The prevalence of PTA in our cohort at 6 months (early PTA) was 51.3% and at 2 years (late PTA) was 36.6%. These results are consistent with previously reported prevalence rates that show that at 6 months posttransplantation, approximately one-half of the patients are anemic[2,6,25] and during the first 5 years posttransplantation, the frequency declines and only approximately one-third are anemic.[5,26]

The predictors found to be significantly associated with early PTA were female sex, lower eGFR at 6 months, and hypochromic RBCs. Previous studies have also found female sex to be an independent predictor for anemia at 6 months as in our study[26] and at 12 months posttransplantation,[12,27] as well as in patients with CKD.[28] A higher eGFR was shown to confer protection against PTA at 12 months.[29] In renal transplant recipients, low renal function was found to be associated with reduced synthesis of erythropoietin by the allograft, thereby resulting in PTA.[30,31] Interestingly, hypochromic RBCs, which are a marker for iron deficiency, were found to be an independent risk factor for mortality in renal transplant recipients.[32]

A novel observation in this study, compared to previous studies, was that the presence of anemia at 6 months posttransplantation (early PTA) predicted late PTA. This fact possibly suggests that better prevention and treatment of early PTA might prevent late PTA. Other predictors for late PTA in our cohort included a low iron level at 2 years posttransplantation, a low triglyceride level as well as low weight before transplantation. Iron deficiency, which is one of the common causes of PTA may be due to postoperative losses, frequent phlebotomy, utilization of the iron stores for enhanced erythropoiesis, and nutritional deficiency.[9,25] A low level of triglycerides has been shown previously by our group to be an independent predictor for emergence of anemia in women in a large cohort of 10,000 adults attending a screening center.[19] Of note, in the same cohort, a low basal metabolic index (BMI) was associated with anemia in women, yet only in a univariate analysis. In another retrospective study of renal transplant recipients, anemic patients had a significantly lower BMI, as in our series.[16] Low BMI has been shown to be associated with anemia in populations other than renal transplant recipients, such as institutionalized elderly patients.[33]

Although iron deficiency is suggested by our findings as the main cause for anemia, chronic inflammation may be an alternate hypothesis. We found no difference in ferritin levels in the anemic versus the nonanemic patients, yet the ferritin levels were relatively high. The iron levels at 2 years, although significantly lower in anemic patients, are not sufficiently low to be responsible for the anemia. Moreover, as ferritin is an acute phase reactant, this may suggest that inflammation has a role in the pathogenesis of anemia. This hypothesis could possibly be strengthened by demonstrating an elevated hepcidin level in the anemic patients, yet this test is currently not available in clinical practice. Interestingly, patients

treated with trimethoprim–sulfamethoxazole had a lower frequency of anemia at 6 months and 2 years and this finding was statistically significant. A possible explanation may be that trimethoprim–sulfamethoxazole actually had a protective role for anemia by its antimicrobial and anti-inflammatory qualities. This again may lend support to the inflammation hypothesis.

A novel finding that was not previously reported in renal transplant recipients is the decline of eGFR with time in patients with anemia. There was a difference of $5.26 \, \text{mL/min/1.73 m}^2$ in eGFR, between 6 months and 2 years. The eGFR in anemic patients declined by $2.26 \, \text{mL/min/1.73 m}^2$, whereas the eGFR increased in the nonanemic patients. Hb level has previously been shown to be an independent risk factor for doubling of serum creatinine or for end-stage renal disease (ESRD) in patients with diabetes in the RENAAL study.[34] Hb was incorporated into a risk score predicting ESRD alone or ESRD with death, together with albuminuria, hypoalbuminemia, and serum creatinine level.[35] Similar results were shown in a cohort of Japanese patients with CKD. A rapid decline of eGFR was strongly associated with anemia.[36] To the best of our knowledge, the finding that Hb is associated with GFR decline was not shown so far in renal transplant recipients. As low serum iron was significantly associated with late PTA, and hypochromia was a risk factor for early PTA, iron deficiency anemia may be partial responsible for the decline in GFR. These observations suggest that early and aggressive treatment of anemia after kidney transplantation might reduce the rate of GFR decline or even the decline in graft function. However, due to the retrospective design of our study, we cannot assume causality between anemia and GFR decline, and this might be only an association. Moreover, CKD is known to be associated with reduced erythropoiesis and anemia, and decreased renal function is associated with reduced synthesis of erythropoietin by the allograft.[22]

Mortality and graft failure rate were higher in patients with early and late PTA. The important findings in our study are that PTA at 2 years was an independent predictor for 4-year mortality, with every increment of 1 g/dL in Hb level, reducing mortality by 22% (HR 0.784, 95% CI 0.616–0.998). In addition, PTA at 2 years was an independent predictor for graft failure, with every increment of 1 g/dL in Hb level, reducing graft failure by 22% (HR 0.775, 95% CI 0.619–0.969, for every increment of 1 g/dL). These findings are consistent with previous reports. In a prospective cohort study of 938 kidney transplant recipients in a single center in Hungary, PTA was also found to be associated with mortality and graft failure at the end of a 4-year follow-up,[13] with a HR of 1.69 (95% CI 1.115–2.560) for mortality. In a large retrospective analysis of 2031 transplant recipients in Austria, anemia was significantly associated with mortality and graft failure, at a median follow-up of 6 years, with every increment of 1 g/dL in Hb level, reducing mortality by 18% (HR 0.82, 95% CI 0.76–0.89).[14] In another retrospective series of 626 transplantation patients in a single-center cohort from Pennsylvania, PTA at 12 months was associated with an increased risk of death (with a HR of 3.0, 95% CI 1.3–6.7).[12]

Nevertheless, other studies have shown conflicting results regarding long-term mortality. In a retrospective European study of 825 renal transplant recipients, anemia was not associated with all-cause mortality with a follow-up of 8 years.[16] In another multicenter prospective cohort of 2102 transplant recipients, Hb levels were not associated with any effect on cardiovascular morbidity and mortality at a follow-up of 5 to 6 years.[17] In a retrospective Chinese study of 887 renal transplantation recipients, PTA at 12 months was not associated

with mortality.[15] Yet, in all of these studies, anemia was significantly associated with graft loss, as in our study.[16,17,29]

Anemia has been associated with mortality in various clinical settings. In a population-based cohort study, anemia was found to be an independent risk factor for all-cause mortality in the general population.[37] Anemia is also associated with increased mortality in the setting of heart failure,[38] acute coronary syndrome,[39] perioperative cardiac surgery,[40] and malignancy.[41] Importantly, anemia is associated with mortality in CKD.[1,42]

Several mechanisms may explain the association between anemia and mortality in renal transplant recipients. Similarly to the CKD population, cardiovascular factors are the leading cause of death in renal transplant recipients.[43] Anemia has been associated with left ventricular hypertrophy and congestive heart failure in renal transplant recipients, and both of these factors, as well as anemia itself, were independent predictors of mortality.[18,44] Iron deficiency, independently of anemia, was also found in a prospective study to be associated with mortality in renal transplant recipients.[45] In our study, markers of iron deficiency (percentage of hypochromic RBCs) were significantly associated with early PTA, and low serum iron was associated with late PTA, which in turn was associated with mortality. Hence, the link between anemia and mortality may possibly be through iron deficiency.

The strengths of our study is the adherence to the acceptable WHO definitions for anemia, which were endorsed by the American Society for Transplantation and differ for men (Hb < 13 g/dL) and women (Hb < 12 g/dL).[21,22] Many prior studies have used a single threshold for both men and women (usually <11–12), thus possibly underestimating the frequency of anemia. In addition, this study assessed PTA at 2 time points after transplantation, unlike most previous studies that studied only 1 time point.

Our study has several limitations. First, this is a retrospective cohort study and as such the information was collected from computerized medical files only and from a single-center database. Therefore, care should be taken in generalizing the results to the entire kidney transplantation population. Second, data regarding erythropoiesis-stimulating agents (ESAs) after transplantation were too scarce to analyze, and this may have possibly affected patient outcomes. However, randomized controlled trials (RCTs) that examined the effect of ESA compared with placebo on graft function did not find a beneficial effect.[46,47] We also lacked data regarding administration of oral or intravenous (IV) iron posttransplantation. In contrast to the abundance of data from RCTs on the efficacy of iron in the CKD population,[48,49] similar studies in transplant recipients are scarce. Our group reported on elevation of Hb levels with IV iron administration after transplantation in a small retrospective study.[50] In contrast to our experience, an RCT comparing oral with a single dose of IV iron after transplantation did not show a difference in time to anemia correction.[51]

4.1. Implication for practice and research

The predictors that we found for PTA suggest that there are several risk groups in which a closer follow-up for early identification of anemia might be warranted—women and decreased eGFR for early PTA, a low triglyceride level, low body weight, a low iron level, and a low Hb level posttransplantation for late PTA. The fact that early PTA predicted late PTA in renal transplantation recipients and late PTA was associated with decline in eGFR, graft failure and mortality have important clinical and therapeutic implications. As anemia is a relatively easily modifiable risk factor, preventable causes for anemia such as iron deficiency should be sought and anemia should be treated as early as possible. Future RCTs should focus on correction of anemia using either iron, ESAs, or both in an effort to improve graft function, graft survival, and patient survival after transplantation. Future research should better elucidate the link between PTA and mortality.

References

[1] Servilla KS, Singh AK, Hunt WC, et al. Anemia management and association of race with mortality and hospitalization in a large not-for-profit dialysis organization. Am J Kidney Dis 2009;54:498–510.

[2] Lorenz M, Kletzmayr J, Perschl A, et al. Anemia and iron deficiencies among long-term renal transplant recipients. J Am Soc Nephrol 2002;13:794–7.

[3] Molnar MZ, Novak M, Ambrus C, et al. Anemia in kidney transplanted patients. Clin Transplant 2005;19:825–33.

[4] Saito S, Fujiwara T, Sakagami K, et al. Anemia following renal transplantation. Transplant Proc 1998;30:3025–6.

[5] Yorgin PD, Scandling JD, Belson A, et al. Late post-transplant anemia in adult renal transplant recipients. An under-recognized problem? Am J Transplant 2002;2:429–35.

[6] Vanrenterghem Y, Ponticelli C, Morales JM, et al. Prevalence and management of anemia in renal transplant recipients: a European survey. Am J Transplant 2003;3:835–45.

[7] Hricik DE. Anemia after kidney transplantation—is the incidence increasing? Am J Transplant 2003;3:771–2.

[8] Jimeno L, Rodado R, Campos M, et al. Iron deficiency—an under-recognized problem in nonanemic and erythrocytic kidney transplant recipients: risks and effects of ACEI and of iron treatment. Transplant Proc 2005;37:1007–8.

[9] Zheng S, Coyne DW, Joist H, et al. Iron deficiency anemia and iron losses after renal transplantation. Transpl Int 2009;22:434–40.

[10] Sun CH, Ward HJ, Paul WL, et al. Serum erythropoietin levels after renal transplantation. N Engl J Med 1989;321:151–7.

[11] Miles AM, Markell MS, Daskalakis P, et al. Anemia following renal transplantation: erythropoietin response and iron deficiency. Clin Transplant 1997;11:313–5.

[12] Imoagene-Oyedeji AE, Rosas SE, Doyle AM, et al. Posttransplantation anemia at 12 months in kidney recipients treated with mycophenolate mofetil: risk factors and implications for mortality. J Am Soc Nephrol 2006;17:3240–7.

[13] Molnar MZ, Czira M, Ambrus C, et al. Anemia is associated with mortality in kidney-transplanted patients—a prospective cohort study. Am J Transplant 2007;7:818–24.

[14] Heinze G, Mitterbauer C, Regele H, et al. Angiotensin-converting enzyme inhibitor or angiotensin II type 1 receptor antagonist therapy is associated with prolonged patient and graft survival after renal transplantation. J Am Soc Nephrol 2006;17:889–99.

[15] Huang G, Wu LW, Yang SC, et al. Factors influencing graft outcomes following diagnosis of polyomavirus-associated nephropathy after renal transplantation. PLoS One 2015;10:e0142460.

[16] Winkelmayer WC, Chandraker A, Alan Brookhart M, et al. A prospective study of anaemia and long-term outcomes in kidney transplant recipients. Nephrol Dial Transplant 2006;21:3559–66.

[17] Schjelderup P, Dahle DO, Holdaas H, et al. Anemia is a predictor of graft loss but not cardiovascular events and all-cause mortality in renal transplant recipients: follow-up data from the ALERT study. Clin Transplant 2013;27:E636–643.

[18] Rigatto C, Parfrey P, Foley R, et al. Congestive heart failure in renal transplant recipients: risk factors, outcomes, and relationship with ischemic heart disease. J Am Soc Nephrol 2002;13:1084–90.

[19] Gafter-Gvili A, Cohen E, Avni T, et al. Predicting the emergence of anemia—A large cohort study. Eur J Intern Med 2015;26:338–43.

[20] Grossman C, Dovrish Z, Koren-Morag N, et al. Diabetes mellitus with normal renal function is associated with anaemia. Diabetes Metab Res Rev 2014;30:291–6.

[21] World Health Organization Na. Report of a WHO scientific group. (WHO Technical Report Series, No 405: 1–40), Geneva, Switzerland 1968.

[22] Kasiske BL, Vazquez M A, Harmon W E, et al. Recommendations for the outpatient surveillance of renal transplant recipients. American Society of Transplantation. J Am Soc Nephrol 2000;11(suppl 15):S1–86.

[23] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.

[24] American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care 2012;35(suppl 1):S64–71.

[25] Teruel JL, Lamas S, Vila T, et al. Serum ferritin levels after renal transplantation: a prospective study. Nephron 1989;51:462–5.

[26] Mix TC, Kazmi W, Khan S, et al. Anemia: a continuing problem following kidney transplantation. Am J Transplant 2003;3:1426–33.

[27] Shibagaki Y, Shetty A. Anaemia is common after kidney transplantation, especially among African Americans. Nephrol Dial Transplant 2004; 19:2368–73.

[28] Howard AD, Moore JJr, Welch PG, et al. Analysis of the quantitative relationship between anemia and chronic renal failure. Am J Med Sci 1989;297:309–13.

[29] Huang Z, Song T, Fu L, et al. Post-renal transplantation anemia at 12 months: prevalence, risk factors, and impact on clinical outcomes. Int Urol Nephrol 2015;47:1577–85.

[30] Besarab A, Caro J, Jarrell BE, et al. Dynamics of erythropoiesis following renal transplantation. Kidney Int 1987;32:526–36.

[31] Winkelmayer WC, Chandraker A. Pottransplantation anemia: management and rationale. Clin J Am Soc Nephrol 2008;3(suppl 2):S49–55.

[32] Winkelmayer WC, Lorenz M, Kramar R, et al. Percentage of hypochromic red blood cells is an independent risk factor for mortality in kidney transplant recipients. Am J Transplant 2004;4:2075–81.

[33] Silva EC, Roriz AK, Eickemberg M, et al. Factors associated with anemia in the institutionalized elderly. PLoS One 2016;11:e0162240.

[34] Keane WF, Brenner BM, de Zeeuw D, et al. The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL study. Kidney Int 2003;63:1499–507.

[35] Keane WF, Zhang Z, Lyle PA, et al. Risk scores for predicting outcomes in patients with type 2 diabetes and nephropathy: the RENAAL study. Clin J Am Soc Nephrol 2006;1:761–7.

[36] Chang WX, Arai S, Tamura Y, et al. Time-dependent risk factors associated with the decline of estimated GFR in CKD patients. Clin Exp Nephrol 2016;20:58–70.

[37] Martinsson A, Andersson C, Andell P, et al. Anemia in the general population: prevalence, clinical correlates and prognostic impact. Eur J Epidemiol 2014;29:489–98.

[38] Ezekowitz JA, McAlister FA, Armstrong PW. Anemia is common in heart failure and is associated with poor outcomes: insights from a cohort of 12 065 patients with new-onset heart failure. Circulation 2003;107:223–5.

[39] Sabatine MS, Morrow DA, Giugliano RP, et al. Association of hemoglobin levels with clinical outcomes in acute coronary syndromes. Circulation 2005;111:2042–9.

[40] Ranucci M, Di Dedda U, Castelvecchio S, et al. Impact of preoperative anemia on outcome in adult cardiac surgery: a propensity-matched analysis. Ann Thorac Surg 2012;94:1134–41.

[41] Caro JJ, Salas M, Ward A, et al. Anemia as an independent prognostic factor for survival in patients with cancer: a systemic, quantitative review. Cancer 2001;91:2214–21.

[42] Foley RN, Parfrey PS. Anemia in predialysis chronic renal failure: what are we treating? J Am Soc Nephrol 1998;9:S82–4.

[43] Ponticelli C, Villa M. Role of anaemia in cardiovascular mortality and morbidity in transplant patients. Nephrol Dial Transplant 2002;17 (suppl 1):41–6.

[44] Rigatto C, Foley R, Jeffery J, et al. Electrocardiographic left ventricular hypertrophy in renal transplant recipients: prognostic value and impact of blood pressure and anemia. J Am Soc Nephrol 2003;14:462–8.

[45] Eisenga MF, Minovic I, Berger SP, et al. Iron deficiency, anemia, and mortality in renal transplant recipients. Transpl Int 2016;29:1176–83.

[46] Aydin Z, Mallat MJ, Schaapherder AF, et al. Randomized trial of short-course high-dose erythropoietin in donation after cardiac death kidney transplant recipients. Am J Transplant 2012;12:1793–800.

[47] Sureshkumar KK, Hussain SM, Ko TY, et al. Effect of high-dose erythropoietin on graft function after kidney transplantation: a randomized, double-blind clinical trial. Clin J Am Soc Nephrol 2012; 7:1498–506.

[48] Shepshelovich D, Rozen-Zvi B, Avni T, et al. Intravenous versus oral iron supplementation for the treatment of anemia in CKD: an updated systematic review and meta-analysis. Am J Kidney Dis 2016;68:677–90.

[49] Fishbane S, Block GA, Loram L, et al. Effects of ferric citrate in patients with nondialysis-dependent CKD and iron deficiency anemia. J Am Soc Nephrol 2017;28:1851–8.

[50] Rozen-Zvi B, Gafter-Gvili A, Zingerman B, et al. Intravenous iron supplementation after kidney transplantation. Clin Transplant 2012; 26:608–14.

[51] Mudge DW, Tan KS, Miles R, et al. A randomized controlled trial of intravenous or oral iron for posttransplant anemia in kidney transplantation. Transplantation 2012;93:822–6.