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Associations among erythropoietic, iron-related, and FGF23 parameters in pediatric kidney transplant recipients

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Abstract

Background—In pediatric kidney transplant recipients, anemia is common and oftentimes multifactorial. Hemoglobin concentrations may be affected by traditional factors, such as kidney function and iron status, as well as novel parameters, such as fibroblast growth factor 23 (FGF23).

Methods—Here, we evaluated associations among erythropoietic, iron-related, and FGF23 parameters in a cohort of pediatric kidney transplant recipients, hypothesizing that multiple factors are associated with hemoglobin concentrations.

Results—In a cross-sectional analysis of 59 pediatric kidney transplant recipients (median (interquartile range) age 16.3 (13.5, 18.6) years, median estimated glomerular filtration rate (eGFR) 67 (54, 87) ml/min/1.73 m^2), the median age-related hemoglobin standard deviation score (SDS) was −2.1 (−3.3, −1.1). Hemoglobin SDS was positively associated with eGFR and calcium, and was inversely associated with erythropoietin (EPO), mycophenolate dose, and total, but not intact, FGF23. In multivariable analysis, total FGF23 remained inversely associated with hemoglobin SDS, independent of eGFR, iron parameters, EPO, and inflammatory markers, suggesting a novel FGF23-hemoglobin association in pediatric kidney transplant patients. In a subset of patients with repeat measurements, only delta hepcidin was inversely associated with delta hemoglobin SDS. Also, delta EPO positively correlated with delta erythroferrone (ERFE), and delta ERFE inversely correlated with delta hepcidin, suggesting a possible physiologic role for the EPO-ERFE-hepcidin axis in the setting of chronic kidney disease (CKD).

Conclusion—Our study provides further insight into factors potentially associated with erythropoiesis in pediatric kidney transplant recipients.

Keywords

Pediatrics; Kidney transplant; Anemia; Hepcidin; Erythroferrone; Fibroblast growth factor 23

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Introduction

In pediatric kidney transplant recipients, anemia is common [1–6] and associated with worse allograft function [5, 6]. The etiology of post-transplant anemia is oftentimes complex and multifactorial. Factors that may affect hemoglobin concentrations include traditional parameters, such as kidney function, iron status, and certain medications, as well as novel parameters, such as fibroblast growth factor 23 (FGF23). In the current study, we evaluated associations among erythropoietic, iron-related, and FGF23 parameters in a cohort of pediatric kidney transplant recipients.

Insufficient iron availability for erythropoiesis is one of the most common causes of anemia. Decreased iron availability may be secondary to absolute iron deficiency or "functional" iron deficiency, where total body iron stores are sufficient, but the iron is sequestered intracellularly and thus unavailable for erythropoiesis. "Functional" iron deficiency, engendering a restrictive erythropoiesis, is mediated by increased levels of hepcidin. Hepcidin is a hormone produced by the liver [7] that acts on multiple cell types, binding to the cell membrane iron exporter ferroportin, causing its internalization and degradation, resulting in decreased iron export from cells into the circulation [8]. Hepcidin affects enterocytes, inhibiting dietary iron absorption; hepatocytes, preventing the mobilization of hepatic iron stores; and hepatic and splenic macro-phages, inhibiting the efflux of recycled iron [8]. In chronic kidney disease (CKD), as glomerular filtration rate (GFR) declines, circulating hepcidin concentrations increase [9, 10], likely contributed to by increased inflammation [11] and decreased renal clearance [7].

In the setting of impaired kidney function, in addition to dysregulated iron metabolism, relative or absolute EPO deficiency may also contribute to anemia. In non-CKD subjects, as hemoglobin concentrations decrease, serum EPO levels progressively increase in a compensatory response [12]. In CKD subjects, serum EPO levels also initially increase as hemoglobin declines; however, as kidney function worsens, EPO levels do not increase sufficiently enough to offset the decrease in hemoglobin, resulting in a relative EPO deficiency [12]. As kidney function continues to decline, in severe CKD, the relative EPO deficiency becomes absolute.

Erythroferrone (ERFE) is a recently characterized hormone that links erythropoiesis and iron metabolism. In response to EPO, ERFE is produced by erythroblasts and acts on the liver to decrease hepcidin production [13, 14]. Thus, ERFE couples erythropoietic stimuli with decreased hepcidin production, increasing iron availability for erythropoiesis. Whether decreased ERFE production in the setting of CKD contributes to increased hepcidin is unknown.

In addition to factors related to the EPO-ERFE-hepcidiniron axis, FGF23 has also recently been linked to anemia. FGF23 is a predominantly bone-derived phosphaturic hormone that regulates serum phosphate concentrations, and increases early in the course of adult and pediatric CKD [15–18]. In animal studies, data suggest that FGF23 may impair

erythropoiesis [19, 20], and in two large human CKD cohorts, higher FGF23 levels were independently associated with prevalent and incident anemia [21, 22].

It is unclear how contributory any or all of these factors may be to post-transplant anemia. Therefore, in the current study, we investigated associations among hemoglobin, EPO, ERFE, hepcidin, iron, FGF23, and other factors in pediatric kidney transplant recipients, hypothesizing that multiple factors are associated with hemoglobin concentrations.

Methods

This retrospective observational study included 59 pediatric kidney transplant patients from the University of California Los Angeles (UCLA) Pediatric Kidney Transplant Immune Monitoring Study. Patients were enrolled from August 2005 to November 2014. This study was approved by the UCLA Institutional Review Board (#11–002375) and conforms with the 1964 Helsinki declaration and its later amendments or comparable ethical standards and the Principles of the Declaration of Istanbul. Informed consent and patient assent, when appropriate, was obtained for all study subjects.

Study data were collected and managed using REDCap electronic data capture tools hosted at UCLA [23, 24]. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing: (1) an intuitive interface for validated data capture; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for data integration and interoperability with external sources.

In this cohort, serum samples were obtained at a median time of 6.7 months post-transplant. In 29 patients, an additional serum sample was obtained at a median time of 16.0 months after the initial sample collection. Demographic, clinical, and biochemical data, including age, sex, race, ethnicity, height, cause of kidney failure, transplant type (deceased/living donor), type of immunosuppression, hemoglobin, hematocrit, creatinine, calcium, and phosphate were collected from chart review. Estimated glomerular filtration rate (eGFR) was calculated using the revised Schwartz equation [25]. Equimolar mycophenolate doses were calculated using the conversion equation 180 mg mycophenolate sodium (Myfortic) = 250 mg mycophenolate mofetil (CellCept) [26].

Serum samples were assayed for iron, ferritin, hepcidin, EPO, ERFE, C-terminal (total) FGF23, and intact FGF23. Colorimetric methods were used to measure serum iron (Genzyme, Cambridge, MA, USA). In-house enzyme-linked immunosorbent assays (ELISA) were used to measure serum ERFE, as previously described [14]. Commercially available ELISA kits were used to measure serum ferritin (Abcam, Cambridge, MA, USA), serum hepcidin (Intrinsic Life Sciences, San Diego, CA, USA), serum EPO (R&D Systems, Minneapolis, MN, USA), serum C-terminal (total) FGF23 (Quidel, San Diego, CA, USA), and serum intact FGF23 (Quidel, San Diego, CA, USA). Whereas the total FGF23 assay detects both full-length, intact FGF23 and C-terminal FGF23 proteolytic fragments, the intact FGF23 assay detects only the full-length form. Serum inflammatory markers (TNF-

α, IL-1β, IL-6, and IL-8) were measured using a magnetic bead kit (EMD Millipore, Darmstadt, Germany), with fluorescence quantified on a Luminex 200TM instrument. Given that normal hemoglobin, hematocrit, phosphate, and total FGF23 concentrations vary by age within the pediatric population, we used published normative data for hemoglobin and hematocrit [27], phosphate [28], and total FGF23 [29] to calculate age-related standard deviation scores (SDS) for these parameters. The SDS, also known as a z-score, is defined as the number of standard deviations an observation is above the mean.

A cross-sectional analysis was performed using data collected from the 59 study subjects and their initial serum parameters. Descriptive data are expressed as numbers and percentages for categorical data, or as medians and interquartile ranges (IQR) for continuous data. Correlations among variables were analyzed using Pearson correlation coefficients; given non-normal data distributions, EPO, ERFE, and FGF23 values were log-transformed prior to analysis. Multivariable linear regression analysis was used to identify associations between independent variables and the dependent variable of hemoglobin SDS. In the subset of 29 subjects in whom two serum samples were collected over time, a longitudinal analysis was performed. Here, correlations among parameter changes over time (delta values) were analyzed using Pearson correlation coefficients. *P*-values <0.05 were considered statistically significant. Statistical analysis was performed using GraphPad Prism 9.0.1 (San Diego, CA, USA).

Results

The cross-sectional analysis included 59 pediatric kidney transplant recipients, with a median (IQR) age of 16.3 (13.5, 18.6) years, assessed at a median time of 6.7 (5.5, 21.0) months post-transplant (Table 1). 61% of study subjects were male, and 69% were white. Nearly all subjects (92%) were on mycophenolate immunosuppression, whereas only 63% were on steroid-based immunosuppression. The median eGFR was 67 (54, 87) ml/min/1.73 m^2, and the median hemoglobin concentration was 12.4 (11.2, 13.3) g/dl. The median hemoglobin SDS was −2.1 (−3.3, −1.1). 95% of the cohort had a hemoglobin concentration below the mean for age (SDS < 0); 80% of the cohort had a hemoglobin concentration at least one standard deviation below the mean for age (SDS < −1); and 51% of the cohort had a hemoglobin concentration at least two standard deviations below the mean for age (SDS < −2). Additional baseline characteristics are shown in Table 1.

In univariable analysis (Table 2), hemoglobin SDS was positively associated with eGFR and calcium, and was inversely associated with log-transformed EPO, log-transformed ERFE, log-transformed total FGF23, and mycophenolate dose (Fig. 1a–e). Hemoglobin SDS was not significantly associated with iron, ferritin, hepcidin, intact FGF23, phosphate, TNF-α, or IL-8. Correlations with IL-1β and IL-6 were not assessed, as most subjects had undetectable values for these cytokines. Correlations with prednisone dose were also not assessed, given the limited number of study subjects on steroid-based immunosuppression. Notably, EPO and ERFE values were highly correlated, suggesting a strong link-age between these hormones.

In multivariable linear regression analysis (Table 3), we assessed independent associations between log-transformed total FGF23 and hemoglobin SDS. In different models, we adjusted for different co-variables. Each model had 10–20 subjects per independent variable (to avoid overfitting), normally distributed residuals, and no evidence of collinearity or multicollinearity. Total FGF23 remained significantly associated with hemoglobin SDS in models adjusted for eGFR; iron parameters; EPO and ERFE; phosphate and calcium; inflammatory markers; or mycophenolate dose.

In the longitudinal analysis, which included 29 subjects (Table 4), the change in hemoglobin SDS over time was inversely associated with the change in hepcidin over time (Fig. 2a), but not changes in other variables. Notably, delta EPO was positively associated with delta ERFE, and delta ERFE was inversely associated with delta hepcidin (Fig. 2b,c). Delta hepcidin was inversely associated with delta iron; however, the association did not reach statistical significance (Fig. 2d).

Discussion

In our cohort of pediatric kidney transplant recipients, we evaluated associations among hemoglobin concentrations, erythropoietic factors, iron-related factors, and FGF23. There was a high prevalence of anemia, consistent with what has been observed in other pediatric kidney transplant studies [1–6]. Interestingly, the parameter most strongly associated with hemoglobin SDS was total FGF23 concentrations, which were inversely associated with hemoglobin SDS. In multiple linear regression analysis, this inverse association remained significant, independent of kidney function, iron parameters, EPO, and inflammation. This result is similar to what has been observed in large adult CKD cohorts, where higher total FGF23 levels were independently associated with both prevalent and incident anemia [21, 22].

We measured plasma FGF23 concentrations using both a C-terminal (total) FGF23 assay and an intact FGF23 assay. The total FGF23 assay detects both intact FGF23 protein and C-terminal FGF23 proteolytic fragments, whereas the intact FGF23 assay detects only full-length FGF23. Intracellular regulation of FGF23 is complex in that FGF23 is regulated at both the transcriptional and post-translational stages. Various local and systemic factors can increase FGF23 mRNA transcription, leading to increased FGF23 translation. However, the degree of intracellular post-translational proteolytic cleavage determines how much intact vs. cleaved FGF23 is secreted from the cell into the circulation. As such, circulating total FGF23 measurements reflect the amount of FGF23 transcription/translation, but circulating intact FGF23 measurements are a reflection of the net effects on FGF23 mRNA transcription and FGF23 post-translational cleavage.

In our cohort, total FGF23 was strongly and independently associated with hemoglobin, but intact FGF23 was not. Inverse associations between total FGF23 concentrations and hemoglobin have been previously observed [21, 22, 30–32]; however, only one of these studies also assessed intact FGF23 [31]. In this study, Bielesz et al. evaluated associations between FGF23 concentrations and hemoglobin in 225 adult CKD patients [31]. After adjusting for eGFR, albumin, gender, and diabetes, total FGF23 but not intact FGF23 was

associated with hemoglobin (total FGF23: β = −0.28, $p = 0.001$; intact FGF23: β = −1.04, $p = 0.057$). However, further adjustment for iron parameters (serum iron, ferritin, transferrin, transferrin saturation, and hepcidin) weakened the association between total FGF23 and hemoglobin (β = −0.15, $p = 0.126$), suggesting that the observed inverse association between total FGF23 and hemoglobin may have been at least partially mediated by iron status. Pre-clinical murine studies have demonstrated that iron deficiency concurrently increases *Fgf23* mRNA transcription and FGF23 post-translational proteolytic cleavage, resulting in cellular secretion of large quantities of FGF23 fragments, increasing circulating concentrations of total FGF23 greatly out of proportion to intact FGF23 [33–36]. Consistent with these findings, in human cohorts, serum iron concentrations are inversely associated with total FGF23 but not intact FGF23 [37–39]. However, in the present study, the inverse association between total FGF23 and hemoglobin remained significant after adjustment for serum iron, ferritin, and hepcidin, suggesting the possible influence of factors unrelated to iron metabolism.

Besides iron deficiency, increased EPO [40–45] and inflammation [35] can both couple increased FGF23 transcription with increased FGF23 post-translational proteolytic cleavage, resulting in markedly increased FGF23 fragment production. Therefore, it is possible that increased EPO and/or inflammation may at least partially mediate inverse associations between total FGF23 levels and hemoglobin. However, in our study and the study by Bielesz et al. [31], the association between total FGF23 and hemoglobin remained significant after adjustment for EPO and inflammatory markers, suggesting that neither EPO nor inflammation had a major influence. Potential mechanisms underlying the inverse association between total FGF23 and hemoglobin remain unclear and require further study.

Our study and the study by Bielesz et al. [31] did not observe significant associations between intact FGF23 concentrations and hemoglobin. However, previous murine studies have demonstrated that intact FGF23 may have a negative effect on erythropoiesis. In wild-type mice injected with recombinant intact FGF23 protein, acute decreases in kidney *Epo* mRNA expression [44], serum EPO concentrations [19], and the number of bone marrow erythroid colonies [19] were observed; such effects could result in lower hemoglobin concentrations. Differences in magnitude and/or duration of intact FGF23 exposure may have contributed to the negative findings in the human cohorts. Whereas the murine studies achieved high circulating concentrations of intact FGF23 (mean values of ~1500 pg/ml) [19, 44], the intact FGF23 concentrations observed in the human cohorts were much lower. Also, whereas the murine studies evaluated the acute effects of high intact FGF23 levels (within 24 h of administration) [19, 44], the human studies were cross-sectional in nature, potentially reflecting both chronic and/or compensatory effects related to interactions between intact FGF23 and erythropoiesis.

Besides total FGF23, other factors were also associated with hemoglobin. EPO and hemoglobin concentrations were inversely correlated. In non-CKD subjects, and in those with mild to moderate CKD, as hemoglobin concentrations decrease, serum EPO levels progressively increase in response [12]. In our cohort with relatively mild kidney dysfunction, this compensatory response likely contributed to the observed inverse association. We also observed a strong positive correlation between serum calcium

and hemoglobin concentrations, and an inverse correlation between serum phosphate and hemoglobin concentrations. Bielesz et al. [31] observed similar results; possible mechanisms contributing to these associations are unclear. Lastly, mycophenolate dose was inversely correlated with hemoglobin concentrations. In kidney transplant recipients, higher mycophenolate exposure is associated with an increased incidence of anemia [46].

In our cohort, we measured factors related to erythropoiesis and iron availability, specifically factors related to the EPO-ERFE-hepcidin-iron axis. In the cross-sectional cohort, we observed a strong positive association between serum EPO and ERFE, consistent with the known stimulatory effect of EPO on ERFE production [13, 14]; however, serum ERFE did not significantly correlate with hepcidin. In previous cross-sectional studies of CKD cohorts, strong associations between EPO and ERFE were observed [47], but associations between ERFE and hepcidin were variable [47–49], possibly contributed to by the multiple interrelated factors that affect hepcidin levels in CKD.

Yet, in the longitudinal cohort, increases in EPO were strongly associated with increases in ERFE, and increases in ERFE were associated with decreases in hepcidin. Decreases in hepcidin were not significantly associated with changes in serum iron, but were significantly associated with increases in hemoglobin. Although ERFE may play a more prominent role in the setting of stress erythropoiesis [50], our findings suggest a possible physiologic role for the EPO-ERFE-hepcidin-iron axis in the setting of CKD.

Our study has limitations and strengths. The primary limitations of our study are that it is retrospective, observational, and has a small sample size. Also, there is incomplete biochemical characterization, as we lack potentially relevant mineral metabolism factors such as vitamin D and parathyroid hormone. The main strength of our study is that, to our knowledge, it is the first study to evaluate associations between FGF23 and hemoglobin in pediatric kidney transplant recipients. We measured both total and intact FGF23 levels, demonstrating that total FGF23, but not intact FGF23, was inversely associated with hemoglobin. This intriguing association was independent of kidney function, iron status, EPO levels, and inflammatory markers, suggesting possible contributions from other underlying factors. Our study is also the first to measure serum ERFE levels in an exclusively pediatric CKD cohort. We observed associations among changes in EPO, EFRE, and hepcidin concentrations, providing more insight into the nature of this recently characterized hormone. Overall, our study highlights novel factors potentially associated with erythropoiesis in pediatric kidney transplant recipients. Future clinical research in this area may focus on evaluating these associations in larger CKD cohorts, and future pre-clinical research may focus on investigating whether therapeutically targeting FGF23 or hepcidin improves anemia in the setting of CKD.

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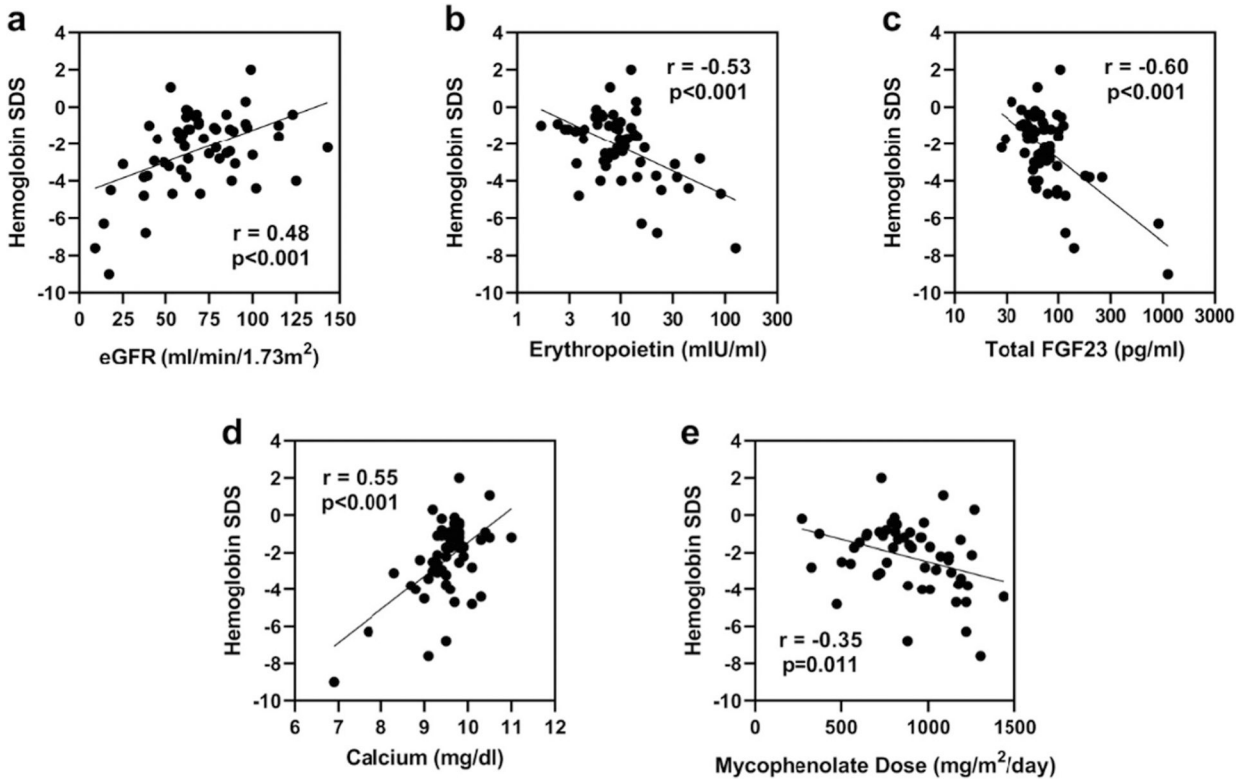

Fig. 1.

Correlations with hemoglobin standard deviation scores (SDS). Presented are the Pearson correlation coefficients for hemoglobin SDS vs. (**a**) estimated glomerular filtration rate (eGFR), (**b**) serum erythropoietin, (**c**) serum total fibroblast growth factor 23 (FGF23), (**d**) serum calcium, and (**e**) mycophenolate dose

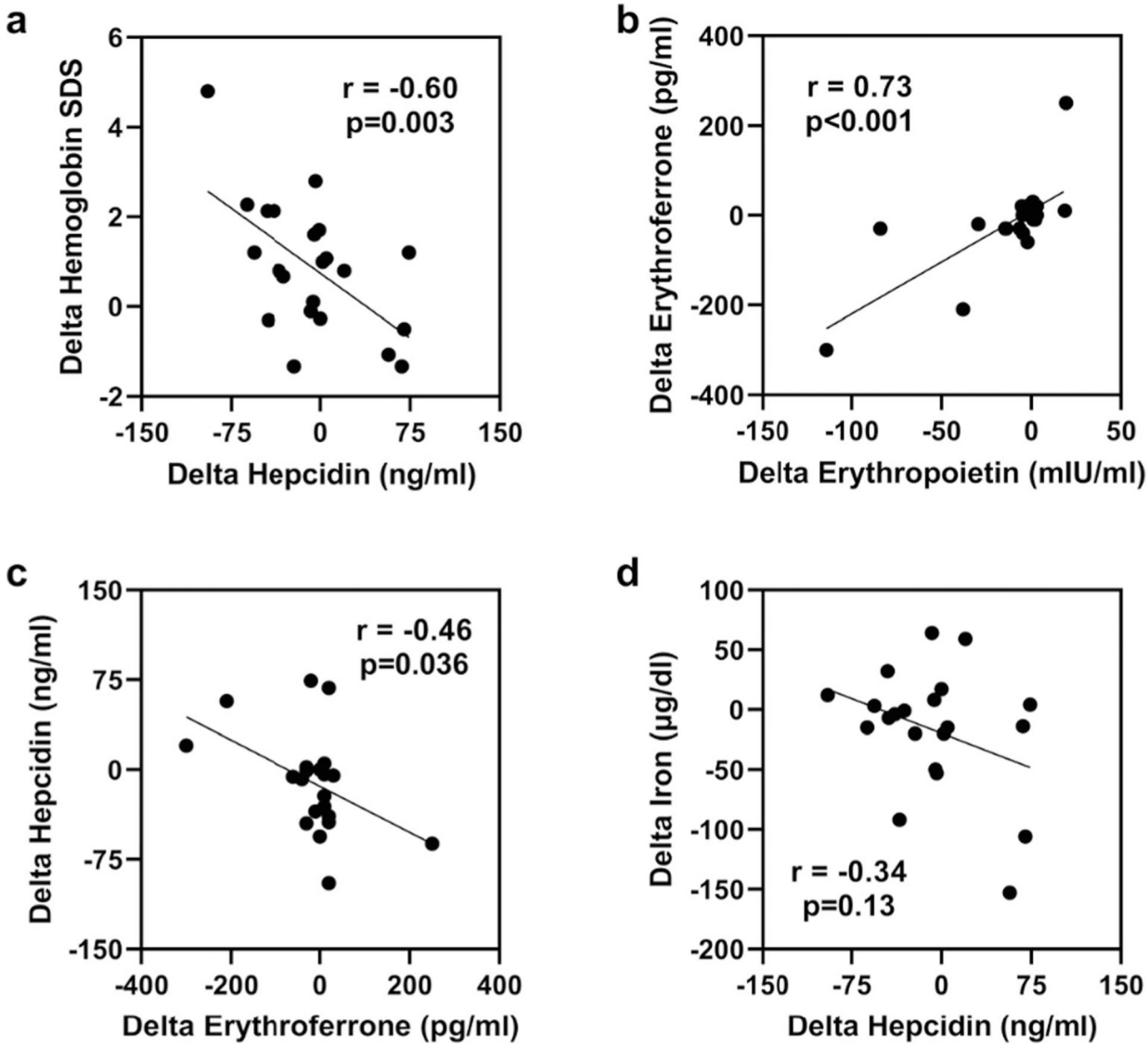

Fig. 2.

Correlations among changes in erythropoietic parameters over time. Presented are the Pearson correlation coefficients for (**a**) delta hemoglobin standard deviation score (SDS) vs. delta hepcidin, (**b**) delta erythroferrone vs. delta erythropoietin, (**c**) delta hepcidin vs. delta erythroferrone, and (**d**) delta iron vs. delta hepcidin

Table 1

Demographic data, clinical characteristics, and baseline serum parameters of the cross-sectional cohort (n = 59). GFR: glomerular filtration rate; SDS: standard deviation score; FGF23: fibroblast growth factor 23

Variable	N (%)/Median (IQR)
Age, years	16.3 (13.5, 18.6)
Sex, male	36 (61%)
Race:	
White	41 (69%)
Asian	4 (7%)
Black	4 (7%)
Other	10 (17%)
Etiology of kidney failure:	
Obstructive uropathy	16 (27%)
Dysplasia	9 (15%)
Focal segmental glomerulosclerosis	8 (14%)
Glomerulonephritis	7 (12%)
Polycystic kidney disease	2 (3%)
Other or unknown	17 (29%)
Deceased donor	36 (61%)
Immunosuppression:	
Prednisone	37 (63%)
Mycophenolate	54 (92%)
Calcineurin inhibitor	57 (97%)
Prednisone dose (mg/kg/day, n = 37)	0.09 (0.07, 0.15)
Mycophenolate Dose (mg/m^2/day, n = 54)	892 (732, 1117)
Other medications:	
Epoetin alfa	11 (19%)
Ferrous sulfate	9 (15%)
Calcium carbonate	3 (5%)
Neutra-Phos	3 (5%)
Epoetin alfa dose (IU/kg/week, n = 11)	299 (131, 466)
Months post transplant	6.7 (5.5,21.0)
Estimated GFR (ml/min/1.73 m^2)	67 (54, 87)
Hemoglobin (g/dl)	12.4 (11.2, 13.3)
Hemoglobin SDS	−2.1 (−3.3, −1.1)
Hematocrit (%)	37.2 (33.6, 40.1)
Hematocrit SDS	−1.9 (−3.3, −1.0)
Iron (μg/dl)	82 (63, 108)
Ferritin (ng/ml)	117 (51, 338)
Hepcidin (ng/ml)	34 (26, 78)
Erythropoietin (mIU/ml)	9.2 (6.5, 14.2)
Erythroferrone (pg/ml)	25 (10, 40)

Variable	N (%)/Median (IQR)
Total FGF23 (RU/ml)	68 (58, 98)
Total FGF23 SDS	0.2 (−0.4, 1.7)
Intact FGF23 (pg/ml)	130 (55, 226)
Phosphate (mg/dl)	4.1 (3.3, 4.6)
Phosphate SDS	−0.9 (−2.0, 0.1)
Calcium (mg/dl)	9.6 (9.3, 9.8)
TNF-α (pg/ml)	9.2 (6.8, 13.4)
IL-10β (pg/ml)	0.0 (0.0, 2.3)
IL-6 (pg/ml)	0.0 (0.0, 4.7)
IL-8 (pg/ml)	12.6 (4.3, 31.8)

Table 2

Pearson correlation coefficients (with *p*-values) for the cross-sectional analysis ($n = 59$, except mycophenolate dose, for which $n = 54$). Boxes shaded orange indicate a statistically significant positive correlation, and boxes shaded blue indicate a statistically significant inverse association. Hb: hemoglobin; SDS: standard deviation score; eGFR: estimated glomerular filtration rate; EPO: erythropoietin; ERFE: erythroferrone; tFGF23: total fibroblast growth factor 23; iFGF23: intact fibroblast growth factor 23

	Hb SDS	Hb	eGFR	Iron	Ferritin	Hepcidin	Log EPO	Log ERFE	Log tFGF23	Log iFGF23	Phosphate	Phosphate SDS	Calcium	TNFα	IL-8	Mycophenolate Dose
Hb SDS																
Hb	**0.90** *<0.001*															
eGFR	**0.48** *<0.001*	**0.34** *0.009*														
Iron	−0.04 0.77	0.02 0.86	0.17 0.22													
Ferritin	−0.19 0.16	−0.12 0.40	0.12 0.36	0.21 0.13												
Hepcidin	−0.10 0.49	−0.09 0.50	0.13 0.34	0.02 0.92	**0.44** *0.001*											
Log EPO	**−0.53** *<0.001*	**−0.52** *<0.001*	−0.20 0.14	−0.06 0.66	−0.05 0.74	−0.17 0.23										
Log ERFE	**−0.36** *0.005*	**−0.29** *0.027*	**−0.30** *0.023*	−0.07 0.61	0.01 0.94	−0.24 0.08	**0.59** *<0.001*									
Log tFGF23	**−0.60** *<0.001*	**−0.53** *<0.001*	**−0.52** *<0.001*	−0.06 0.66	0.10 0.45	0.05 0.74	0.25 0.06	**0.27** *0.040*								
Log iFGF23	−0.04 0.80	0.14 0.31	−0.19 0.17	−0.05 0.74	−0.17 0.22	0.03 0.82	−0.17 0.21	0.03 0.81	0.19 0.08							
Phosphate	−0.22 0.10	**−0.34** *0.009*	−0.07 0.63	−0.06 0.67	−0.09 0.53	0.05 0.74	**0.28** *0.035*	0.11 0.40	0.05 0.73	**−0.33** *0.013*						
Phosphate SDS	−0.15 0.24	−0.23 0.08	−0.18 0.18	−0.06 0.65	−0.12 0.36	−0.08 0.57	0.26 0.06	0.02 0.86	0.03 0.80	**−0.28** *0.034*	**0.87** *<0.001*					

	Hb SDS	Hb	eGFR	Iron	Ferritin	Hepcidin	Log EPO	Log ERFE	Log tFGF23	Log iFGF23	Phosphate	Phosphate SDS	Calcium	TNFα	IL-8	Mycophenolate Dose
Calcium	**0.55** *<0.001*	**0.55** *<0.001*	**0.38** *0.003*	−0.08 0.57	−0.23 0.09	−0.11 0.44	−0.25 0.06	**−0.34** *0.009*	**−0.55** *<0.001*	−0.11 0.43	**−0.30** *0.020*	−0.22 0.09				
TNFα	−0.22 0.10	−0.13 0.32	−0.18 0.17	0.03 0.84	**0.32** *0.015*	0.16 0.23	−0.12 0.39	0.13 0.31	**0.31** *0.017*	0.09 0.51	−0.03 0.80	−0.11 0.40	**−0.26** *0.048*			
IL-8	−0.03 0.85	0.07 0.63	−0.22 0.09	0.17 0.23	0.05 0.71	−0.10 0.45	−0.11 0.41	0.08 0.53	0.19 0.14	0.24 0.07	−0.14 0.31	−0.10 0.43	−0.15 0.25	**0.53** *<0.001*		
Mycophenolate Dose	**−0.35** *0.011*	**−0.31** *0.022*	−0.12 0.39	−0.08 0.58	0.23 0.11	0.09 0.54	**0.28** *0.046*	0.08 0.58	0.25 0.07	0.09 0.53	−0.12 0.41	−0.15 0.28	−0.14 0.33	0.15 0.29	0.19 0.18	

Table 3

Multivariable linear regression analysis to evaluate independent associations between log-transformed total FGF2 3 (independent variable) and hemoglobin SDS (dependent variable). VIF: variance inflation factor; FGF23: fibroblast growth factor 23; eGFR: estimated glomerular filtration rate; EPO: erythropoietin; ERFE: erythroferrone

Model	Independent variables	Coefficient	*p*-value	VIF	R^2
1	**Log Total FGF23**	**−3.496**	**<0.001**	**1.37**	0.40
	eGFR	0.016	0.07	1.37	
2	**Log Total FGF23**	**−4.354**	**<0.001**	**1.02**	0.40
	Iron	−0.004	0.52	1.06	
	Ferritin	−0.001	0.50	1.34	
	Hepcidin	−0.002	0.77	1.26	
3	**Log Total FGF23**	**−2.930**	**0.001**	**1.06**	0.42
	Log EPO	**−1.924**	**0.006**	**1.61**	
	Log ERFE	−0.199	0.61	1.55	
4	**Log Total FGF23**	**−3.152**	**<0.001**	**1.45**	0.45
	Phosphate SDS	−0.104	0.48	1.06	
	Calcium	**0.945**	**0.021**	**1.52**	
5	**Log Total FGF23**	**−4.303**	**<0.001**	**1.11**	0.38
	TNF-α	−0.020	0.40	1.48	
	IL-8	0.008	0.24	1.39	
6	**Log Total FGF23**	**−3.301**	**<0.001**	**1.07**	0.30
	Mycophenolate Dose	−0.002	0.06	1.07	

Table 4

Pearson correlation coefficients (with *p*-values) for the longitudinal analysis ($n = 29$, except for delta mycophenolate dose, for which $n = 22$). Boxes shaded orange indicate a statistically significant positive correlation, and boxes shaded blue indicate a statistically significant inverse association. Hb: hemoglobin; SDS: standard deviation score; eGFR: estimated glomerular filtration rate; EPO: erythropoietin; ERFE: erythroferrone; tFGF23: total fibroblast growth factor 23; iFGF23: intact fibroblast growth factor 23

	Delta Hb SDS	Delta Hb	Delta eGFR	Delta Iron	Delta Ferritin	Delta Hepcidin	Delta EPO	Delta ERFE	Delta tFGF23	Delta iFGF23	Delta Phosphate	Delta Phosphate SDS	Delta Calcium	Delta TNFα	Delta IL-8	Delta Mycophenolate Dose
Delta Hb SDS																
Delta Hb	**0.94** *<0.001*															
Delta eGFR	0.23 0.23	0.34 0.07														
Delta Iron	0.26 0.20	0.27 0.17	**0.52** *0.006*													
Delta Ferritin	0.10 0.66	0.10 0.67	0.28 0.21	0.08 0.73												
Delta Hepcidin	**−0.60** *0.003*	**−0.50** *0.017*	0.03 0.89	−0.34 0.13	0.08 0.73											
Delta EPO	0.04 0.87	0.04 0.86	**−0.44** *0.033*	−0.18 0.42	−0.03 0.91	−0.32 0.15										
Delta ERFE	0.23 0.23	0.26 0.17	−0.16 0.40	0.00 0.99	0.34 0.13	**−0.46** *0.036*	**0.73** *<0.001*									
Delta tFGF23	−0.14 0.46	−0.28 0.15	**−0.44** *0.018*	−0.09 0.65	**−0.45** *0.036*	−0.35 0.11	**0.52** *0.009*	0.30 0.13								
Delta iFGF23	−0.15 0.47	−0.08 0.70	0.09 0.67	0.06 0.79	−0.28 0.22	0.28 0.22	0.18 0.42	−0.09 0.69	0.13 0.27							
Delta Phosphate	0.05 0.81	0.04 0.86	−0.18 0.39	−0.09 0.68	−0.29 0.24	**−0.53** *0.024*	0.25 0.29	0.06 0.77	0.17 0.43	−0.09 0.70						
Delta Phosphate SDS	0.04 0.84	0.03 0.88	−0.21 0.32	−0.10 0.66	−0.28 0.26	**−0.52** *0.026*	0.27 0.25	0.08 0.71	0.17 0.43	−0.09 0.71	**1.00** *<0.001*					

	Delta Hb SDS	Delta Hb	Delta eGFR	Delta Iron	Delta Ferritin	Delta Hepcidin	Delta EPO	Delta ERFE	Delta tFGF23	Delta iFGF23	Delta Phosphate	Delta Phosphate SDS	Delta Calcium	Delta TNFα	Delta IL-8	Delta Mycophenolate Dose
Delta Calcium	0.36 0.05	**0.42** *0.022*	0.20 0.30	−0.09 0.65	−0.10 0.66	0.01 0.96	−0.13 0.56	−0.17 0.38	−0.32 0.09	−0.15 0.48	0.19 0.36	0.18 0.39				
Delta TNFα	0.03 0.86	0.13 0.50	−0.08 0.68	0.05 0.80	−0.02 0.93	−0.05 0.83	0.07 0.73	0.00 1.00	0.16 0.40	0.19 0.38	0.00 1.00	0.00 0.99	0.09 0.65			
Delta IL-8	0.10 0.61	0.17 0.37	−0.07 0.73	0.06 0.78	−0.05 0.81	−0.12 0.59	0.09 0.67	0.02 0.91	0.11 0.58	0.05 0.82	0.22 0.29	0.23 0.27	0.12 0.53	**0.77** *<0.001*		
Delta Mycophenolate Dose	−0.34 0.12	−0.30 0.18	−0.08 0.73	−0.02 0.94	−0.11 0.70	−0.20 0.47	**0.59** *0.009*	0.31 0.17	0.07 0.76	0.08 0.76	−0.10 0.68	−0.10 0.69	−0.24 0.27	0.06 0.78	0.03 0.90	