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Hepcidin and Risk for Anemia in CKD: A Cross-sectional and Longitudinal Analysis in the CKiD Cohort

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Abstract

Background—Hepcidin, a key iron regulatory protein, is elevated in patients with chronic kidney disease (CKD). Its role in the development and progression of the anemia of CKD in children remains poorly defined.

Methods—Cross-sectional and longitudinal study in children aged 1–16 years with stage 2–4 CKD in the Chronic Kidney Disease in Children (CKiD) cohort (n=133) with hepcidin measured at baseline and hemoglobin (HGB) measured annually in follow-up. Anemia was defined as HGB < 5th percentile for age/sex OR treatment with an erythropoiesis stimulating agent (ESA).

Results—Hepcidin levels correlated negatively with glomerular filtration rate (GFR) ($r=-0.22$, $p=0.01$) and positively with ferritin ($r=0.67$, $p<0.001$). At the lower end of the GFR spectrum at baseline (10th percentile, 27.5 ml/min/1.73m²), higher hepcidin was associated with a 0.87 g/dL decrease in HGB during follow-up (95% CI -1.69, -0.05 g/dL, $p=0.038$). At higher GFR percentiles there was no significant association between baseline hepcidin and HGB during follow-up. Among 90 non-anemic subjects at baseline, 23.3% developed incident anemia. In subjects with GFR the median, a higher hepcidin level was associated with an increased risk for incident anemia (at 10th %ile GFR, HR 3.471, 95% CI 1.228, 9.810, $p=0.019$; at 25th %ile GFR HR 2.641, 95% CI 1.213, 5.750, $p=0.014$; at 50th %ile GFR, HR 1.953, 95% CI 1.011, 3.772, $p=0.046$). Among subjects with GFR in the 75th percentile or above, incrementally higher baseline hepcidin was not associated with increased anemia risk.

Conclusions—Higher hepcidin levels are associated with a decreased HGB and an increased risk for incident anemia, and this association is most significant among subjects with lower GFR.

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Keywords

Chronic kidney disease (CKD); children; anemia; hepcidin

Introduction

The identification of hepcidin as a key iron-regulatory protein has helped to clarify the mechanisms underlying the anemia of chronic kidney disease (CKD), beyond erythropoietin deficiency alone. Hepcidin, encoded by the *HAMP* gene and produced in the liver, regulates both intestinal iron absorption and body iron distribution through its post-translational suppression of cell-membrane expression of ferroportin, the sole cellular iron exporter [1]. Hepcidin is initially synthesized as an 84 amino acid prepropeptide, which is then cleaved by the prohormone convertase furin to form the active 25 amino acid form [1]. Hepcidin binding causes internalization and degradation of ferroportin, which results in down-regulation of dietary iron absorption via intestinal enterocytes, and inhibits the release of stored iron from reticuloendothelial cells [2–4]. In this way hepcidin mediates iron-restricted erythropoiesis by preventing the utilization of absorbed or stored iron for erythropoiesis by the bone marrow, and it plays a significant role in the development of both the anemia of CKD and resistance to therapy with erythropoiesis stimulating agents (ESA) [5–7]. Hepcidin levels have been found to be elevated in both adults and children with chronic kidney disease and on dialysis [4,8,9]. Hepcidin bound to ferroportin is co-internalized and degraded in lysosomes, while the major mechanism for the clearance of circulating hepcidin is free glomerular filtration and subsequent proteolysis in the proximal tubule [10]. Previous studies have noted an inverse relationship between serum hepcidin and estimated glomerular filtration rate (GFR) [8,11,12].

Known regulators of hepcidin production in humans include hypoxia, erythropoiesis/anemia, and iron status [13]. In addition, novel regulators of hepcidin production continue to come to light including vitamin D, which has recently been shown to suppress expression of *HAMP* *in vitro* and to decrease circulating hepcidin levels in healthy volunteers [14]. Inflammation, and in particular the inflammatory cytokine IL-6, is also a well-established stimulus for hepcidin production [3,15,16]. CKD in adults is widely recognized as a pro-inflammatory state, but although elevated C-reactive protein (CRP) levels have been associated with increased hepcidin in some adults with CKD, correlations between hepcidin and CRP or other markers of inflammation have not been consistently demonstrated [13,15,16]. Among children with CKD enrolled in the Chronic Kidney Disease in Children (CKiD) cohort study, the prevalence of elevated CRP levels is not particularly high, nor does CRP appear to increase with decreasing GFR as had been noted in adults [17]. Impaired GFR, however, is widely evident in this population, and it remains to be determined which of these possible mechanisms may contribute more significantly to increased hepcidin levels.

Hepcidin is likely a modifiable mediator of the anemia of CKD in children, and a potential target for future therapies, including the treatment of ESA-resistant anemia beyond ESA dose escalation. However, further characterization of the relationship between hepcidin levels and hemoglobin in children with CKD, as GFR declines, is necessary to clarify its

specific contribution to anemia. Our objective in this analysis was to determine the distribution of hepcidin levels in a cohort of children with non-dialysis CKD, and to test the hypothesis that hepcidin would be associated with hemoglobin (HGB), anemia status, GFR and other clinical characteristics in this population. Additionally, we tested whether hepcidin levels at baseline might prospectively identify changes in HGB and incidence of anemia over time.

Methods

Study Population and Design

We performed cross-sectional and longitudinal analyses using data from the first cohort (enrolled from January 2005 – August 2009) of the CKiD Study, an observational prospective cohort study of CKD in children conducted at 50 centers in North America [18]. Institutional Review Board approval was obtained at each participating center. Eligibility criteria for the CKiD study included: age 1–16 years, estimated GFR 30–90 ml/min/1.73 m² calculated using the original Schwartz equation [19], and no prior organ transplantation. The cross-sectional analysis was limited to participants who had HGB and hepcidin measurements performed at a baseline (V2) visit, and who were not receiving iron supplementation at baseline (n=133), as treatment with iron raises hepcidin levels. Longitudinal HGB data was available annually after the baseline visit (V3 and V4, max follow-up time 2.5 years), and a total of 137 subjects (including 3 subjects missing a HGB at baseline) had hepcidin measurements at baseline along with HGB levels available during follow-up and were included in the regression analyses.

Study Variables

Blood samples were collected using standardized techniques. HGB and mean corpuscular volume (MCV) were measured locally at clinical sites as part of a complete blood count. Proficiency testing surveys published by the College of American Pathologists demonstrate coefficients of variation of 1–2% for the various instruments performing Hgb measurements [20]. In the cross-sectional analysis, anemia was defined as a HGB less than the 5th percentile for age and sex based on normative population data [21,22] or treatment with an ESA. For the longitudinal analysis in subjects without anemia at baseline, incident anemia was defined as either the initiation of treatment with an ESA or HGB less than the 5th percentile for age and sex at follow-up. Hepcidin-25, the biologically active form of the peptide, was measured in previously frozen serum via a validated competitive enzyme-linked immunosorbent assay (ELISA) in a single laboratory (Intrinsic LifeSciences, La Jolla, CA) [23]. As the distribution of the hepcidin measurements was severely skewed to the right, hepcidin was log-transformed with base 10 [$\log(\text{hepcidin})$]. Frozen serum samples for hepcidin measurement were shipped in two separate batches with varied total freezing time (time from sample collection to testing) between batches, resulting in slightly different distributions of $\log(\text{hepcidin})$ for each assay group (median [IQR] $\log(\text{hepcidin})$ 4.2 [3.6, 5.1] for batch 1 and 3.7 [3.1, 4.3] for batch 2). In statistical comparison of the two shipped batches, the only sample characteristic that was significantly different between batches was total freezing time (median [IQR] 2.32 [2.05, 2.78] years for batch 1 vs. 3.54 [3.02, 4.11] years for batch 2, $p<0.001$). Therefore, in order to eliminate confounding by freezing time,

log(hepcidin) was adjusted against freezing time and assay groups by taking the residuals from the regression of the hepcidin levels on freezing time and assay groups [adj(hepcidin)]. All other laboratory assays were performed at the CKiD central laboratory (University of Rochester, Rochester, NY). Measured iron status markers included serum iron and serum ferritin, but were only available in a subset of subject for this analysis (n=63). GFR was measured directly by iohexol plasma disappearance over 5 hours (iGFR). In 13 of 133 subjects who were missing a baseline iGFR, GFR was calculated using an estimating equation derived from the CKiD study and shown to be precise (eGFR) [24]. Information regarding age, sex, race, and ethnicity was collected using standardized forms. A self-report of primary CKD diagnosis was categorized as glomerular or non-glomerular. A list of the specific CKD diagnoses and their glomerular/non-glomerular classification has been previously published [25]. Data on anemia treatment included self-reported use of ESA.

Statistical Analysis

Demographic and clinical characteristics were summarized overall and by hepcidin quartile (< 21, 21–42, 42–66, and ≥ 66 ng/mL) using median and interquartile range for continuous variables, and percentage (%) and frequency (n) for categorical variables. Differences in level or frequency of characteristics by hepcidin quartile were tested using Jonckheere-Terpstra tests for continuous variables and Cochran-Armitage trend tests for categorical variables. All variables were tested for a 2-sided alternative. The correlations between log(hepcidin) and ieGFR, HGB, and ferritin were assessed using scatterplots and Spearman's rank correlation testing. As the association between log(hepcidin) and ferritin was noted not to be linear (Figure 1, panel c), the ranks of log(hepcidin) and ferritin were obtained by arranging and ranking the values from smallest to largest before fitting a regression line.

Given that the measurements from the same individual at different visits were correlated, generalized estimating equation (GEE) modeling was utilized to examine the association of hepcidin with change in Hgb level over time (V2, V3, V4), adjusting for within-subject correlations. Models were also adjusted for covariates likely to influence HGB including age, race, sex, underlying cause of CKD, ESA use, years from baseline visit and ieGFR. A significant interaction between hepcidin and ieGFR was noted, suggesting that level of ieGFR modifies the association between hepcidin and HGB, and this interaction term was included in the GEE models as well. All covariates were fixed at baseline except for ESA use. Cox proportional hazards regression modeling was used to examine the association of baseline hepcidin with incident anemia during the follow up period, also adjusted for the covariates likely to be associated with risk for anemia as in the GEE model (except years from baseline visit). Additionally, a significant interaction between age and underlying cause of CKD was noted, and this interaction was also included in the Cox model.

Multiples of each regression type were performed with ieGFR values centered at various specified ieGFR percentiles, in order to examine the association between adj(hepcidin) and HGB/risk for anemia at varying levels of ieGFR. ieGFR was centered at each percentile by subtracting the percentile from the original ieGFR. To allow for differing effects of hepcidin on HGB as ieGFR varies, models contain centered ieGFR and its interaction with

adj(hepcidin). Thus, the regression coefficients of the adj(hepcidin) represent the effects of adj(hepcidin) on HGB at the particular value of ieGFR at which the original ieGFR is centered.

Results

One-hundred thirty-three children not receiving iron supplementation at baseline were included in the cross-sectional analysis. Median [IQR] baseline age of subjects was 12.64 [9.06, 15.79] years, and 58.39% of subjects were male. The underlying causes of CKD were listed as congenital anomalies of the kidneys and urinary tract in 57.8% (n=77), cystic kidney disease in 4.5% (n=6), focal segmental glomerulosclerosis in 8.3% (n=11), hemolytic uremic syndrome in 3.0% (n=4), other glomerular disease in 6.8% (n=9), and other non-glomerular disease in 19.6% (n=26) of subjects. The median baseline ieGFR was 44.65 [35.82, 55.73] ml/min/1.73m². Median baseline HGB was 12.8 [11.9, 13.9] g/dL and median baseline hepcidin was 41.7 [21.6, 66.4] ng/mL. Baseline demographic and clinical characteristics of the cohort by hepcidin quartile at baseline are presented in Table 1. Higher hepcidin quartile was associated with lower ieGFR. There was no significant difference in HGB level by hepcidin quartile. The highest prevalence of anemia was noted in the highest hepcidin quartile, but this difference did not reach statistical significance. Similarly, there was a trend for an increase in serum ferritin with increasing hepcidin quartile, but this did not reach statistical significance, likely due to smaller sample size in the subcohort of subjects with iron data available. Finally, the highest prevalence of ESA use was noted in subjects in the lowest hepcidin quartile.

Figure 1 displays the baseline correlations between log(hepcidin) and ieGFR, HGB, and ferritin. A strong negative correlation between log(hepcidin) and ieGFR was noted ($r = -0.22$, $p = 0.01$), but there was no significant correlation between log(hepcidin) and HGB at baseline. A significant positive rank-based correlation between log(hepcidin) and ferritin was also noted ($r = 0.67$, $p < 0.001$).

Table 2 displays the results of the GEE regression analysis of the association between adj(hepcidin) at baseline and change in HGB level during follow-up. In this analysis we found that the association of baseline hepcidin with change in HGB over time depended on baseline level of ieGFR in a non-linear fashion. At the lower end of the ieGFR spectrum (e.g. 10th percentile ieGFR of 27.5 ml/min/1.73m²), increasing baseline hepcidin level is associated with an estimated 0.87 g/dL decrease in HGB during follow up. At the 25th percentile of ieGFR, the direction of the HGB decrease is similar, but the magnitude is decreased and significance is lost. At higher levels of ieGFR, changes in HGB with increasing hepcidin are negligible.

Results of a survival analysis examining risk for incident anemia in 90 subjects without anemia at baseline are shown in Table 3. Twenty-one subjects (23.3%) developed incident anemia during the follow-up period. Again, we found that the association of baseline hepcidin and risk for incident anemia during the follow-up period was modified by baseline level of ieGFR. In subjects with ieGFR 50 ml/min/1.73m² or below, higher hepcidin level was significantly associated with an increased risk for the development of incident anemia,

with the point estimates for the hazard ratio increasing as ieGFR decreases. Conversely, among subjects with baseline ieGFR in the 75th percentile or higher, incrementally higher baseline hepcidin was not associated with increased risk for anemia.

Discussion

The adverse effects of anemia of CKD in children with regard to increased risk for mortality and hospitalization have been well documented [26,27], as has the high prevalence of treatment-resistant anemia [28]. Our study demonstrates that in a cohort of children with CKD, serum hepcidin levels are negatively correlated with GFR and positively correlated with serum ferritin, consistent with results of previous cross-sectional studies in both adults and children with CKD [5,8,11,29]. In addition, we have shown that among those at the lower end of the GFR spectrum, higher baseline hepcidin levels are associated with a decrease in HGB over time and with an increased risk for incident anemia. This is the first time, to our knowledge, that this association has been observed in a prospective pediatric CKD cohort. This supports a potential role for hepcidin as a modifiable mediator in the development and progression of the anemia of CKD, particularly in children with severely decreased kidney function.

Our results also suggest that GFR, as measured by the plasma disappearance of iohexol, is a powerful effect modifier in the association between hepcidin and HGB/anemia risk. This indicates that the role of hepcidin in the development, progression, and response to treatment of anemia in CKD is complex, and that elevated hepcidin concentrations may not be a primary determinant of HGB level in early CKD.

Cross-sectional studies have demonstrated that hepcidin levels are consistently higher in children and adults with CKD and on dialysis compared to healthy controls [5,8]. Normal serum hepcidin reference values have been difficult to define, in large part due to the various types of available assays (including ELISA and mass spectrometry), limiting comparison of levels across studies [30,31]. In a large cohort of healthy adults, median levels were found to be 21.8 ng/mL in men, 11.4 ng/mL in women aged < 55 years, and 23.7 ng/mL in women 55 and older [32]. In a study by Zaritsky et al., using the same ELISA assay performed in the same laboratory utilized in our analysis, the median hepcidin level was noted to be 25.3 ng/mL in healthy pediatric controls and 72.9 ng/mL in adult controls [8]. In 48 children with stage 2–4 CKD, the median hepcidin level was noted to be 127.3 ng/mL, considerably higher than the median 41.7 ng/mL noted in our cohort. However, this difference may be explained in part by differences in the underlying cause of CKD between cohorts, with 46% of Zaritsky et al.'s cohort having glomerulonephritis or focal segmental glomerulosclerosis as the primary cause of CKD, compared to only 18% with underlying glomerular disease in our cohort [8]. We did note a non-significant trend for an increasing proportion of subjects with underlying glomerular disease in the higher hepcidin quartiles in the current analysis. Among the four main regulators of hepcidin production (hypoxia, erythropoiesis, inflammation and iron status), inflammation may be a relatively more significant promoter in patients with underlying glomerular disease compared to those with congenital forms of CKD, although the specific mechanisms for this remain to be clarified [13,15,33]. Interestingly, the lowest hepcidin quartile in our analysis (< 21 ng/mL) also demonstrated

the highest proportion of subjects on ESA therapy. ESAs have been shown to be potent inhibitors of hepcidin expression (move references to the end of this clause), a response that under normal physiologic circumstances likely serves to improve iron availability for erythropoiesis which might suggest that patients on ESAs with low hepcidin levels could effectively absorb enteral iron supplements [11,15].

Hepcidin production is known to be upregulated by iron loading/treatment.³¹ Indeed, we restricted the current analysis to subjects not treated with iron after noting that serum hepcidin levels were consistently, systematically higher in CKiD subjects treated with iron compared to untreated subjects, independent of other clinical factors including GFR. By limiting our analysis to iron-naïve subjects, we intended to clarify the natural history of hepcidin levels and their association with HGB levels and anemia incidence. Given the central role hepcidin plays in regulating iron metabolism, our results confirming a strong positive correlation between serum hepcidin and ferritin levels support the observation that serum ferritin level is not a clinically useful indicator of utilizable stored iron. In a previous analysis done within the CKiD cohort study, we showed that among children with GFR < 60 ml/min/1.73m², ferritin levels > 100 ng/mL are actually associated with significantly lower HGB values compared to children with ferritin levels below 100 ng/mL [34]. An association between high ferritin and low HGB has also been observed in an international cohort of children on peritoneal dialysis [27]. Ferritin levels in children at the lower end of the GFR spectrum may be more a surrogate marker of hepcidin activity, and perhaps of active inflammation, than a useful indicator of available stored iron. Hepcidin measurement may be more useful than traditional markers of iron status including ferritin as an indicator of iron-restricted erythropoiesis, in which case anemia may be minimally responsive to treatment with enteral iron supplements and/or ESAs.

In light of clinical trial data in adults regarding the adverse effects of using escalating ESA doses to treat resistant anemia [35,36], along with recent observational evidence in children on dialysis that higher ESA doses are associated with an increased risk for mortality [27,37], there is a need for adjunctive anemia therapies with the potential to delay the need for ESA initiation or allow effective use of lower doses to maintain adequate HGB levels and avoid the need for red blood cell transfusions. Elevated hepcidin levels may be useful to identify anemic patients who may respond to IV iron administration, a task for which traditional iron indices are of limited utility [38]. However, IV iron therapy also carries risk for adverse events including iron overload and increased oxidative stress, in addition to the more acute administration complication of anaphylaxis, limiting its efficacy [21]. Targeted alternative anemia therapies which manipulate the hepcidin pathway to decrease its production or activity are potentially attractive options, which could enhance absorption of enteral iron, decrease the need for intravenous iron administration, and mobilize intracellular iron stored as ferritin for effective erythropoiesis. There are a variety of interventions currently under investigation which may decrease hepcidin production, neutralize the active protein itself, or block the effects of hepcidin binding to ferroportin [39–41]. RNA interference and gene silencing technologies to inhibit hepcidin gene transcription are under development in animal models, but are still quite remote from clinical application in humans [39–41]. Anti-hepcidin monoclonal antibodies and L-RNA aptamers (Spiegelmers) which can inhibit hepcidin activity and overcome erythropoietin resistance in animal models have

been developed, with some currently in Phase I human clinical trials [35–37]. Interventions to prevent the endocytosis of ferroportin in response to binding by hepcidin are also in pre-clinical development [39,40]. It is still too early to predict which, if any, of these potential therapies is most likely to improve anemia management in conjunction with our current, widely-utilized treatments.

Our study has several strengths, including the use of data from the CKiD study, the largest prospective cohort study of children with CKD in North America. GFR was measured directly in the majority of subjects and in those with no measured GFR, we used an estimating equation derived from the CKiD study and shown to be precise [24,42]. Data were collected using standardized forms and protocols, and biomarkers were measured at single, centralized laboratories. Nevertheless, there are limitations worth mentioning. We restricted this analysis to children not receiving iron treatment at baseline so as not to confound the relationship between hepcidin and HGB, but acknowledge that these untreated children are likely a select population. Serum ferritin levels were available for only a subset of subjects. We did not have access to data regarding markers of inflammation such as CRP, as this was not measured in CKiD subjects at the visits at which concurrent hepcidin and hemoglobin were measured, and thus were not able to assess the relative contribution of inflammation to hepcidin levels in this cohort. We did not have access to parathyroid hormone levels to assess hyperparathyroidism as a risk for anemia in this analysis. Additionally, we did not have data on vitamin D levels available to examine a potential association with hepcidin in this population. While we did find a significant association between increasing hepcidin and risk for anemia, intra-individual and diurnal variability in hepcidin levels has been reported [7,43]. Given that blood samples for hepcidin were not collected at standardized times from individuals across the study, a single hepcidin measurement may not reflect the peak levels experienced by the subject and thus may underestimate the effect size of the association between hepcidin and HGB. Thus, determining the predictive value of a single hepcidin measurement requires further investigation. However, the potential causal association between hepcidin and risk for anemia over time is strengthened in this analysis due to the prospective, longitudinal study design.

In conclusion, we found that among those at the lower end of the GFR spectrum, higher baseline hepcidin levels are associated with a decrease in HGB over time and with an increased risk for incident anemia in children with CKD. This suggests a role for hepcidin in the progression of the anemia of CKD as GFR declines, with the implication that clinical interventions to target the hepcidin-ferroportin axis may be an ESA-independent option for treatment. Further study is needed to clarify whether decreased filtration and degradation of hepcidin vs. increased hepcidin production in response to inflammatory or other stimuli is the primary factor responsible for increased hepcidin levels, and to determine whether these factors may vary by the underlying cause of CKD.

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Figure 1.

Scatter plots of (a) log-transformed serum hepcidin vs GFR (mL/min/1.73m²); (b) HGB (g/dL) vs log(hepcidin) among 133 children; (c) log serum hepcidin (ng/mL) vs serum ferritin (ng/mL); (d) ranks of hepcidin and ferritin among 63 children.

Table 1

Baseline Characteristics by Hepcidin Quartile (n=133)

Characteristic Median (IQR) or % (n)	Baseline Hepcidin (ng/ml)				
	< 21 (n=32)	[21–42] (n=36)	[42–66] (n=31)	> 66 (n=34)	p-value
Age (years)	12.2 (6.8, 15.8)	13.9 (9.8, 16.7)	11.4 (8.7, 15.1)	12.8 (10.3, 14.7)	0.6438
Male	65.6% (21)	61.1% (22)	48.4% (15)	55.9% (19)	0.2866
African-American	12.5% (4)	11.1% (4)	29.0% (9)	11.8% (4)	0.6069
ieGFR (ml/min/1.73m ²)	48.4 (37.1, 59.6)	49.3 (30.3, 58.8)	43.0 (37.6, 51.4)	41.5 (29.7, 51.3)	0.0314
% with Glomerular Dz	9.4% (3)	19.4% (7)	22.5% (7)	20.6% (7)	0.2337
Hgb (g/dL)	13.0 (12, 13.9)	13.2 (12.1, 14)	12.9 (12, 14.1)	12.3 (11.7, 13.6)	0.3834
% Anemic ^a	31.3% (10)	30.6% (11)	19.4% (6)	47.1% (16)	0.2936
MCV ^β (fL)	82.9 (80.8, 87.9)	84.4 (81.0, 87.3)	82.0 (80.1, 85.6)	84.1 (80.3, 86.0)	0.9198
Ferritin (ng/mL)*	13.5 (12, 19.3)	26.5 (16, 44)	32.0 (25.8, 48.5)	50.0 (41, 72)	0.5382
Serum Iron (μg/dL)*	79.0 (55, 86.8)	68.5 (59.3, 91)	82.5 (69.8, 93.8)	72.0 (66, 92.5)	0.2670
TSAT ^ε (%)	21 (14.5, 26.3)	21 (18.3, 28.5)	27 (21.5, 32.0)	25 (22.0, 29.5)	0.5210
ESA Use	12.5% (4)	5.6% (2)	0.0% (0)	11.7% (4)	0.7604

^aHemoglobin < 5th percentile for age/sex OR treatment with an ESA;^βMean corpuscular volume;^εTransferrin saturation;

* n=63

Table 2

Generalized estimating equation regression of baseline adj(hepcidin) and associated longitudinal change in HGB centered at multiple levels of ieGFR (n=137)*

ieGFR (ml/min/1.73m ²) centered at:	Change in HGB (g/dL) per 1 unit increase in adj(hepcidin)	95% CI	P-value
10 th percentile (27.5)	−0.869	(−1.69, −0.05)	0.038
25 th percentile (36.5)	−0.596	(−1.264, 0.073)	0.081
Median/50 th percentile (46)	−0.305	(−0.881, 0.271)	0.299
Mean (47.6)	−0.257	(−0.827, 0.313)	0.377
75 th percentile (55.8)	−0.005	(−0.594, 0.583)	0.986
90 th percentile (70.9)	0.455	(−0.339, 1.250)	0.261

* Model adjusted for age, race, gender, underlying cause of CKD (glomerular vs. non-glomerular), ESA use, years from baseline, ieGFR, and the interaction between hepcidin and ieGFR

Table 3

Survival analysis demonstrating risk for incident anemia^a by baseline hepcidin level centered at multiple levels of iGFR (n=90)*

ieGFR (ml/min/1.73m ²) centered at:	Hazard Ratio [per 1 unit increase in adj(hepcidin)]	95% CI	P-value
10 th percentile (35.50)	3.471	(1.228, 9.810)	0.019
25 th percentile (42.84)	2.641	(1.213, 5.750)	0.014
Median/50 th percentile (50.94)	1.953	(1.011, 3.772)	0.046
Mean (52.31)	1.856	(0.956, 3.605)	0.068
75 th percentile (61.71)	1.308	(0.543, 3.153)	0.550
90 th percentile (72.37)	0.880	(0.234, 3.305)	0.849

^a Anemia defined as HGB less than the 5th percentile for age/sex OR initiation of treatment with an ESA

* Model adjusted for age, race, gender, underlying cause of CKD (glomerular vs. non-glomerular), ieGFR, the interaction between hepcidin and ieGFR, and the interaction between underlying cause of CKD and age