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Association between common iron store markers and hemoglobin in children with chronic kidney disease

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Abstract

Background—Serum ferritin and transferrin saturation (TSAT) are used to assess iron status in children with chronic kidney disease (CKD), but their sensitivity in identifying those at risk of lower hemoglobin (HGB) values is unclear.

Methods—We assessed the association of iron status markers (ferritin, TSAT, and serum iron) with age- and gender-related HGB percentile in mild-to-moderate CKD in 304 children in the

Chronic Kidney Disease in Children (CKiD) Study. Standardized HGB percentile values were examined by KDOQI-recommended ferritin (< 100 ng/ml) and TSAT (< 20 %) thresholds. Regression tree methods were used to identify iron status markers and clinical characteristics most associated with lower HGB percentiles.

Results—The cohort was 62 % male, 23 % African American, and 12 % Hispanic, median age 12 years, and median HGB 12.9 g/dl. 34 % had low TSAT and 93 % low ferritin as defined by KDOQI. Distribution of HGB percentile values was lower in those with ferritin > 100 ng/ml, while TSAT < 20 % was associated with only modest increase in HGB percentile. In regression tree analysis, lower glomerular filtration rate (GFR), serum iron < 50 μ g/dl and ferritin > 100 ng/ml were most strongly associated with lower HGB percentile.

Conclusions—The level of GFR was significantly associated with HGB. Higher serum ferritin was associated with lower HGB in this cohort. Low serum iron in the context of normal/increased ferritin and low HGB may be a useful indicator of iron-restricted erythropoiesis.

Keywords

Chronic kidney disease; Hemoglobin; Iron deficiency; Anemia

Introduction

Although the most common cause of anemia in healthy children is iron deficiency (defined by microcytosis and low serum iron, ferritin and transferrin saturation (TSAT)), the etiology of anemia in children with chronic kidney disease (CKD), and the relative contribution of iron deficiency, is more complex [1]. While erythropoietin deficiency unquestionably plays an important role, iron-restricted erythropoiesis (including absolute iron deficiency, impaired iron-trafficking/iron sequestration, and functional iron deficiency in which available iron supply is insufficient for bone marrow production of red blood cells), is remarkably common, and can confound the interpretation of clinically obtained markers of iron status [2, 3]. It is clear that hemoglobin (HGB) declines as glomerular filtration rate (GFR) declines in this population [4]. Decreased HGB in CKD is associated with adverse clinical outcomes, including decreased quality of life, increased risk of cardiovascular disease and CKD progression, and increased risk of hospitalization and mortality [5–11]. The 2006 Kidney Disease Outcomes Quality Initiative (KDOQI) anemia guidelines recommend measurement of iron status markers to assess the contribution of iron deficiency to anemia [12]. They also suggest that in those treated with erythropoiesis stimulating agents (ESA), serum ferritin > 100 ng/ml and TSAT < 20 % should be maintained in order to ensure an adequate supply of iron for erythropoiesis [12]. However, serum ferritin can be a marker of conditions unrelated to iron storage, such as inflammation, malnutrition, or infection, and cannot be used in isolation to indicate adequate access to stored iron [13]. In children not receiving ESAs, marker-based clinical guidance for assessing iron availability is lacking and target values for iron markers are often extrapolations of the published guidelines for ESA-treated patients.

In this report, we describe the distribution of iron status markers in a large cohort of children with non-dialysis CKD who were not treated with iron supplementation or ESAs, and describe how markers of iron status at levels recommended by KDOQI are associated with HGB values in this group. Additionally, we characterize the association between markers of iron status and HGB levels in the context of renal insufficiency, with the aim of identifying measures that may help to define the contribution of iron deficiency to the anemia seen in children with CKD.

Methods

Study population and design

This is a cross-sectional analysis using data from the Chronic Kidney Disease in Children (CKiD) Study, an observational prospective cohort study of CKD in children conducted at 47 centers in North America [8]. 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 [14], and no prior organ transplantation.

The current analysis was restricted to data collected at the first visit at which a participant had iron status markers measured; in most cases this was either the baseline or first annual follow-up visit. The analysis was further limited to children not receiving treatment for anemia (self-reported iron supplements or ESA). Additionally, children missing HGB, race, height, weight, GFR, or urine protein: creatinine ratio were excluded.

Study variables

Blood samples were collected using standardized techniques. Measured iron status markers included serum iron, serum ferritin, and total iron-binding capacity (TIBC). All assays were performed at the CKiD central laboratory (University of Rochester, Rochester, New York). TSAT was calculated as: serum iron/TIBC $\times$ 100. HGB was 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 [4]. Gaussian standard z-scores and percentiles of HGB were calculated using published race-, age-, and sex-specific HGB means and standard deviations, thus removing the variability in HGB associated with these characteristics [15].

GFR was measured directly by iothexol plasma disappearance over 5 h; when a measured GFR was not available, GFR was estimated using equations developed by CKiD investigators [16, 17]. 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 [18]. Height and weight were calculated as the average of three measurements and body mass index (BMI) was calculated as weight/height², measured in units of kg/m². BMI percentiles for age and sex were defined using standard U.S. growth charts. Overweight was defined as a BMI percentile 85 to <95; obese as a BMI percentile $\geq$ 95 [19]. First-morning urine total protein (mg/dl) and creatinine (mg/dl) concentrations were measured by the CKiD central laboratory using a Bayer Advia 2400 analyzer. A urine protein-to-creatinine ratio (uP/C) of 0.2 to 2.0 was classified as significant proteinuria; uP/C > 2.0 was classified as nephrotic.

Statistical analysis

Demographic and clinical characteristics were summarized overall and by KDOQI CKD stage (GFR $\geq$ 60 [stage 2], 45–59 [stage 3a], 30–44 [stage 3b], and <30 [stage 4] ml/min/1.73 m²) using median and interquartile range (IQR) for continuous variables and percentage (%) and frequency (*n*) for categorical variables. Differences in level or frequency of characteristics by CKD stage were tested using Kruskal–Wallis tests for continuous variables and Fisher's exact test for categorical variables.

Side-by-side boxplots of serum iron, serum ferritin, and TSAT by CKD stage were generated to describe the distribution of these markers. Proportions of children not achieving recommended KDOQI ferritin and TSAT values for ESA-treated subjects (ferritin $\geq$ 100 mg/

ml, TSAT 20 %) were calculated [11]. The lower limit of normal for serum iron was defined as 50 µg/dl as KDOQI does not supply a CKD-specific target value [15].

Side-by-side percentile plots were used to describe the distribution of HGB z-scores by recommended KDOQI ferritin and TSAT status (ferritin 100 vs. 100 mg/ml, TSAT 20 % vs. 20 %). Shading was used to highlight the proportion of subjects with HGB less than the 5th percentile for age, sex, and race, a standard clinical cutoff for identifying anemia in children. Assuming ferritin and TSAT to be accurate markers of iron stores, and HGB level in CKD subjects to be correlated to iron availability, we hypothesized that children with higher ferritin and higher TSAT would have higher HGB levels for age, sex, and race. Likewise, we hypothesized that a smaller proportion of children with high ferritin and high TSAT, respectively, would have HGB levels less than the 5th percentile for age, sex, and race.

Regression tree methods were used to identify clinical profiles (combinations of characteristics and iron status marker levels) most strongly associated with age-, sex-, and race-specific HGB percentile (as a continuous outcome). Regression trees were chosen for the analysis because they provide a ranking of predictors (i.e., strength of association) and naturally explore interactions and nonlinear relationships between factors. Where linear regression is limited to a single predictive formula applied to all variables in the model, recursive partitioning is able to account for multiple variables, which interact with each other in complicated, non-linear ways.

Branches in the regression tree were determined by identifying, out of a set of binary variables, the best predictor of HGB percentile. Specifically, partitions of the data at each step were determined by the binary variable with the largest absolute value *t* statistic from two-sample Student's *t* tests of HGB z-scores (following standard binary recursive partitioning methodology) [20]. Further branching of the regression tree occurred based on the remaining set of predictors until the following stopping rule was satisfied: either fewer than ten individuals remained in the branch or the most strongly associated variable in the remaining set resulted in a *t* statistic with $p > 0.15$. Groups produced by the branching process with distinct clinical profiles (defined by the branching path), but similar HGB percentile levels were combined using a recursive amalgamation algorithm [21]. Specifically, using a two-sample *t* test of the HGB z-score, groups with unique clinical profiles were combined if the *t* statistic was not significant at $\alpha = 0.01$ level, with the most similar HGB z-score groups being combined first. The following binary predictors were evaluated: GFR with cut-points at 30, 45, and 60 ml/min/1.73 m², glomerular vs. non-glomerular CKD diagnosis, serum ferritin with cut-points at 20, 50, and 100 ng/ml, serum iron with cutpoints at 50, 75, and 100 µg/dl, TSAT with cutpoints at 20 % and 30 %, BMI percentile with cutpoints at 85 and 95, and proteinuria as normal vs. elevated, as well as nephrotic vs. non-nephrotic. The predicted HGB percentiles for the final groups were taken as the median HGB percentile across individuals in each final group.

The robustness of the clinical profiles for defining distinct groups in terms of average HGB percentile was tested using a separate validation data set comprised of later measurements (~1 year later) of HGB and iron markers in a subset of the participants that remained iron supplement- and ESA-naïve. Children in the validation set were classified based on their combination of clinical characteristics and iron marker levels (at the time point of the later measurement) and the branching paths of the regression tree. The average HGB percentile values in each group after classification were compared to each other as well as to those from the original data set used for development.

All analysis was performed using SAS 9.2 statistical software (©2002–08, SAS Institute Inc., Cary, NC). Boxplot figures were produced using S-Plus 8.0 statistical software (©2007, Insightful Corp., Seattle, WA).

Results

Table 1 displays demographic and clinical characteristics of 304 iron- and ESA-naïve patients enrolled in the CKiD cohort, overall and stratified by CKD stage. Overall, median [IQR] age was 12 [8, 15] years, 62 % (187) were male, 23 % (70) were African American, and 12 % (37) were Hispanic. Median HGB was 12.9 [12.1, 13.9] g/dl, and median race-, age-, and sex-standardized HGB percentile was the 31st [6th, 74th]. While the four CKD stage groups did not differ by age, sex, underlying cause of CKD, or BMI percentile, significant differences were seen in race, HGB, HGB percentile, and proteinuria. Specifically, the stage 2 group (GFR 60 ml/min/1.73 m²) had a higher proportion of African American children (38 %) compared to the other groups ($p = 0.003$). Both HGB and HGB percentile values decreased with decreasing GFR. Urine protein/creatinine ratio increased consistently with decreasing GFR ($p < 0.001$).

Overall, median serum iron, ferritin, and TSAT were 75 [55, 99] µg/dl, 33 [22, 50] ng/ml, and 24 % [17, 32], respectively (Table 1). Nineteen percent of children had low serum iron (<50 µg/dl). Using KDOQI guidelines for pre-dialysis, ESA-treated children with CKD, 34 % had low TSAT (<20 %) while 93 % had low ferritin (<100 ng/ml). Figure 1 provides side-by-side boxplots of iron marker distributions by CKD stage. Only serum ferritin showed an association with GFR, trending toward higher levels as GFR decreased ($p = 0.01$). The proportion of children below KDOQI-recommended TSAT and ferritin targets did not differ by CKD stage. HGB percentile was positively correlated with serum iron level (0.21, $p < 0.01$) and TSAT (0.14, $p = 0.01$), and negatively correlated with ferritin (−0.14, $p = 0.01$).

Figure 2 shows the distribution of HGB z-scores among those above and below KDOQI recommended thresholds of ferritin and TSAT, respectively. The 21 children with ferritin 100 ng/ml had *lower* HGB z-scores ($p = 0.01$) and a *larger* proportion with HGB less than the 5th percentile (shaded in gray) compared to the 283 children with ferritin <100 ng/ml (48 vs. 22 %, $p = 0.01$ for difference). Comparing children with TSAT <20 vs. 20 %, the overall distributions of HGB z-scores were similar ($p = 0.10$), with a slightly higher proportion of children with TSAT <20 % having HGB levels less than the 5th percentile (29 vs. 20 %, respectively; $p = 0.09$ for difference). Because these associations did not support our hypothesis that ferritin and TSAT are accurate reflections of available iron for HGB production, we utilized regression tree analysis to more richly explore the relationship between HGB and iron store markers in the context of anemia treatment-naïve CKD subjects.

Figure 3 displays results of the regression tree analysis identifying combinations of iron status marker levels and clinical characteristics associated with age-, sex-, and race-standardized HGB z-scores and percentiles; GFR, serum iron, and serum ferritin were found to be significantly associated with HGB percentile. Three distinct groups were identified, based on a comparison of HGB z-score distributions in the nine final branching paths of the regression tree. The “low” HGB group ($n = 60$) had a median HGB percentile of 4 (by definition, anemic) (IQR: [2, 9]) and consisted of children with the following three clinical profiles: GFR <30 ml/min/1.73 m² ($n = 34$), GFR between 30 and 60 ml/min/1.73 m² coupled with serum ferritin 100 ng/dl ($n = 11$), or a GFR between 30 and 45 coupled with a serum iron <50 µg/dl and a serum ferritin <100 ng/dl ($n = 15$). The “middle” HGB group ($n = 175$) had median HGB percentile of 31 (IQR: [22, 42]) and consisted of children with the following three clinical profiles: a GFR between 45 and 60 coupled with a serum iron <50

$\mu\text{g/dl}$ and serum ferritin $<100 \text{ ng/dl}$ ($n = 18$), a GFR between 30 and 60 with serum ferritin $<100 \text{ ng/dl}$ and serum iron $>50 \mu\text{g/dl}$ ($n = 138$), or a GFR >60 with serum iron $<50 \mu\text{g/dl}$ ($n = 19$). The “high” HGB group ($n = 69$) had median HGB percentile of 69 (IQR: [57, 79]) and consisted entirely of children with GFR >60 and serum iron $>50 \mu\text{g/dl}$.

Of the 304 children in the regression tree training data set, 225 had HGB and a complete profile of clinical variables (iron, GFR, proteinuria, CKD diagnosis) at a follow-up visit. Overall, the validation set had slightly higher HGB percentile levels (median: 37th percentile, IQR: 20, 45). Of the 225 test records, 51 had a variable profile that classified them to the “low” HGB percentile group with a median [IQR] HGB percentile of 7 [2, 16]. There were 126 records with a “middle” marker profile with a median HGB percentile of 45 [35, 56]. The remaining 48 records had a variable profile that classified them into the “high” HGB percentile group with a median HGB percentile of 63 [47, 77]. Thus, with respect to HGB percentile, the ranking between the three groups was maintained in the validation dataset and the difference in HGB z-score between groups was significant (“low” vs. “middle”, $p < 0.01$) or marginally significant (“middle” vs. “high”, $p = 0.08$).

Discussion

In clinical practice, where the ability to directly quantify body iron stores by bone marrow iron staining is limited by practical considerations, serum levels of ferritin and TSAT are commonly used to assess body iron stores. In general, serum ferritin is considered a marker of stored iron and TSAT an indicator of the amount of iron available for transportation to the bone marrow for erythropoiesis. While KDOQI recommendations specify target thresholds for these markers during ESA treatment in children with CKD, target levels for ESA-untreated CKD patients are lacking. The prevalence of iron deficiency, defined by TSAT $<20\%$ and low serum ferritin levels, among children with early stage CKD has been reported to be high (42 % in a small cohort by Baracco et al.), but this too has been determined without a gold standard assessment of stored iron [22]. If ferritin and TSAT values are accurate reflections of stored iron, and if in turn iron deficiency is a primary etiologic factor for lower HGB in children with early stage CKD, we hypothesized that subjects with lower HGB values would also demonstrate lower values for markers of iron status. However, we found that children with ferritin below the KDOQI threshold did not have lower HGB values. Likewise, the TSAT threshold provided only moderate discrimination, with marginally higher HGB z-scores in those with TSAT $>20\%$. The use of thresholds from ESA-treated CKD populations did not clearly identify untreated children with lower HGB z-scores. Thus, we thus sought to identify other, more consistent, clinical factors associated with HGB in this population.

Our analysis suggests that in children with moderate to advanced non-dialysis CKD, the distribution of HGB is determined by a much more complicated constellation of factors, with iron markers comprising only one component. It is well established that the etiology of the anemia of CKD is multifactorial, with decreased production of erythropoietin a primary factor as GFR declines, but iron-restricted erythropoiesis is also an important contributor [3]. A single threshold for any iron marker cannot account for the myriad factors that influence iron marker levels. Moreover, serum ferritin is an unreliable indicator of stored iron in the setting of acute inflammation as it is an acute phase reactant [13]. Thus, while low serum ferritin nearly always indicates iron deficiency, high serum ferritin cannot rule it out; rather than indicating adequate amounts of accessible stored iron, it may be more consistent with inflammation-mediated iron sequestration/impaired iron trafficking [23]. In our analysis, ferritin was found to be inversely associated with both HGB and GFR. A recent study by Fishbane et al. in adults with non-dialysis CKD similarly found that serum ferritin

level was not associated with risk for anemia [24]. Very few subjects demonstrated ferritin values consistent with iron deficiency as defined in healthy children.

Using regression tree analysis, a methodology suited to exploring complex relationships between variables, we examined the relationship between age-, sex-, and race-standardized HGB levels and iron marker levels, as well as other clinical markers of CKD. We showed that low GFR, low serum iron, and high serum ferritin were the variables most strongly associated with lower HGB levels. While in the absence of gold standard bone marrow iron studies for defining iron deficiency anemia we are unable to provide specific new thresholds to define “iron-deficiency” in CKD, our findings do suggest that definitions may need to be constructed in the context of CKD stage. For example, while the combination of a GFR ≥ 60 ml/min/1.73 m² and serum iron <50 μ g/dl was a marker profile associated with a “middle” level HGB percentile, a GFR <30 ml/min/1.73 m², independent of serum iron or ferritin level, served as a marker profile associated with “low” HGB percentile. Among children in the lower-middle range of GFR (30–45 ml/min/1.73 m²) with ferritin <100 ng/ml, serum iron <50 μ g/dl was associated with a “low” HGB. However, among children with slightly higher GFR (45–60 ml/min/1.73 m²), a similar iron and ferritin profile was associated with “middle” level HGB. The regression tree analysis consistently demonstrated that markers of iron status became less strongly associated with HGB percentiles as GFR declined. This suggests that at decreasing levels of GFR, iron-restricted erythropoiesis due to iron sequestration may become more prevalent, such that the availability of accessible iron dwindles.

Among the iron markers evaluated, we found serum iron to be the most strongly associated with HGB percentile. Serum iron, a direct measurement of circulating iron available for utilization by erythroid precursors, may be a reliable indicator of iron availability in general [25]. The KDOQI guidelines do not indicate goal values for serum iron, likely due to concerns including the lack of data supporting the use of serum iron as a marker of iron status in CKD, possible diurnal variation in iron levels, and the oxidative stress associated with excess circulating iron [25]. Rather, guidelines suggest measuring ferritin to assess iron stores and TSAT to assess adequacy of iron for erythropoiesis; caution in using IV iron agents in those with significantly elevated serum ferritin is also recommended [12]. As the molecular mechanisms of iron homeostasis, including the role of increased serum hepcidin in iron sequestration, have been clarified, our understanding of iron-restricted erythropoiesis has been advanced [23]. In the presence of normal or elevated serum ferritin, low serum iron values may serve to identify patients in whom iron sequestration is a significant contributor to anemia. In a prospective observational study of more than 1,200 adults maintained on hemodialysis examining the role of low serum iron levels on clinical outcomes, patients with serum iron in the lowest quartile (<45.5 μ g/dl) had a mortality rate twice as high as seen in other quartiles [25]. Serum iron was also inversely associated with mortality and hospitalization, independent of demographics, markers of nutrition and inflammation such as albumin and ferritin, hemoglobin, and doses of ESA and IV iron [25]. The results of our cross-sectional analysis suggest that children with more advanced CKD may need higher levels of serum iron to maintain HGB as GFR declines, likely due to increasing iron-sequestration. While it has been shown in healthy children that increased BMI is associated with lower serum iron levels, BMI percentile was not significantly associated with HGB percentile in this analysis [26].

This analysis does have limitations, including its cross-sectional design, which limits the ability to interpret the relationships between clinical variables and HGB as causal. In addition, we restricted the analysis to children not receiving ESA or iron treatment so as not to confound the relationship between iron markers and HGB, but acknowledge that these untreated children are likely a select population; further analyses will need to be performed

to assess these relationships among treated children. Endogenous erythropoietin levels are not available for subjects in CKiD, and we were thus unable to examine the association of this factor with HGB percentile, although erythropoietin levels are likely closely related to GFR, which we did include. We were not able to include markers of inflammation, including C-reactive protein, in our analysis. As such, we were unable to investigate whether higher serum ferritin levels were correlated with laboratory evidence of inflammation, a finding that would have offered confirmatory evidence to support the concept of higher serum ferritin as primarily an indicator of inflammation-associated iron-sequestration. Finally, we are unable to compare biomarkers of iron status to a gold standard measure due to the practical limitations of obtaining bone marrow iron staining in this population.

The main strength of our study is that it was conducted using data from the largest prospective cohort study of children with CKD. GFR was directly measured in the majority of subjects; in those with no measured GFR available, we used an estimating equation derived from the CKiD study and shown to be precise [16, 17]. Data were collected using standardized forms and protocols and biomarkers were measured at a single central laboratory.

In conclusion, we have demonstrated that while the majority of children in the CKiD cohort fail to meet the KDOQI recommended values for serum ferritin, very few of them meet the ferritin criteria for absolute iron deficiency. Our results suggest that, in a cohort of children with non-dialysis dependent CKD, ferritin may be more a marker of inflammation than a marker of stored iron. Serum iron was identified as strongly associated with HGB level, suggesting that it may be a more specific marker of iron-sequestration than either ferritin or TSAT. Low serum iron in the context of normal or high ferritin (>100) and anemia may be a useful indicator of iron-restricted erythropoiesis, guiding specific therapeutic interventions targeted toward improving iron delivery and utilization. Further study should confirm the clinical utility of serum iron as a target in anemia management, and determine if higher targets for serum iron as GFR declines are associated with higher hemoglobin levels, until iron sequestration can be directly targeted therapeutically.

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References

1. Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the united states. *JAMA*. 1997; 277:973–976. [PubMed: 9091669]
2. Koshy SM, Geary DF. Anemia in children with chronic kidney disease. *Pediatr Nephrol*. 2008; 23:209–219. [PubMed: 17245602]

3. Atkinson MA, Furth SL. Anemia in children with chronic kidney disease. *Nat Rev Nephrol.* 2011; 7:635–641. [PubMed: 21894183]
4. Fadrowski JJ, Pierce CB, Cole SR, Moxey-Mims M, Warady BA, Furth SL. Hemoglobin decline in children with chronic kidney disease: baseline results from the Chronic Kidney Disease in Children prospective cohort study. *Clin J Am Soc Nephrol.* 2008; 3:457–462. [PubMed: 18235140]
5. Mitsnefes MM, Kimball TR, Kartal J, Witt SA, Glascock BJ, Khoury PR, Daniels SR. Progression of left ventricular hypertrophy in children with early chronic kidney disease: 2-year follow-up study. *J Pediatr.* 2006; 149:671–675. [PubMed: 17095341]
6. Schaefer F. Cardiac disease in children with mild-to-moderate chronic kidney disease. *Curr Opin Nephrol Hypertens.* 2008; 17:292–297. [PubMed: 18408481]
7. Amaral S, Hwang W, Fivush B, Neu A, Frankenfield D, Furth S. Association of mortality and hospitalization with achievement of adult hemoglobin targets in adolescents maintained on hemodialysis. *J Am Soc Nephrol.* 2006; 17:2878–2885. [PubMed: 16943308]
8. Furth SL, Cole SR, Moxey-Mims M, Kaskel F, Mak R, Schwartz G, Wong C, Muñoz A, Warady BA. Design and methods of the Chronic Kidney Disease in Children (CKiD) prospective cohort study. *Clin J Am Soc Nephrol.* 2006; 1:1006–1015. [PubMed: 17699320]
9. Wuhl E, Schaefer F. Therapeutic strategies to slow chronic kidney disease progression. *Pediatr Nephrol.* 2008; 23:705–716. [PubMed: 18335252]
10. Filler G, Mylrea K, Feber J, Wong H. How to define anemia in children with chronic kidney disease? *Pediatr Nephrol.* 2007; 22:702–707. [PubMed: 17216248]
11. Staples AO, Wong CS, Smith JM, Gipson DS, Filler G, Warady BA, Martz K, Greenbaum LA. Anemia and risk of hospitalization in pediatric chronic kidney disease. *Clin J Am Soc Nephrol.* 2009; 4:48–56. [PubMed: 19056614]
12. Initiative Kidney Disease Outcomes Quality, Foundation National Kidney. KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease. *Am J Kidney Dis.* 2006; 47(Suppl 3):S11–S145. [PubMed: 16678659]
13. Kalantar-Zadeh K, Lee GH. The fascinating but deceptive ferritin: to measure it or not to measure it in chronic kidney disease? *Clin J Am Soc Nephrol Suppl.* 2006; 1:S9–S18.
14. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics.* 1976; 58:259–263. [PubMed: 951142]
15. Hallowell JG, van Assendelft OW, Gunter EW, Lewis BG, Najjar N, Pfeiffer C. Centers for Disease Control and Prevention, National Center for Health Statistics. Hematological and iron-related analytes-reference data for persons aged 1 year and over: United States, 1988–94. *Vital Health Stat.* 2005; 247:1–156.
16. Schwartz GJ, Furth S, Cole SR, Warady B, Muñoz A. Glomerular filtration rate via plasma iothexol disappearance: Pilot study for chronic kidney disease in children. *Kidney Int.* 2006; 69:2070–2077. [PubMed: 16612328]
17. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol.* 2009; 20:629–637. [PubMed: 19158356]
18. Wong CS, Pierce CB, Cole SR, Warady BA, Mak RH, Benador NM, Kaskel F, Furth SL, Schwartz GJ. CKiD Investigators. Association of proteinuria with race, cause of chronic kidney disease, and glomerular filtration rate in the Chronic Kidney Disease in Children Study. *Clin J Am Soc Nephrol.* 2009; 4:812–819. [PubMed: 19297612]
19. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Mei Z, Wei R, Curtin LR, Roche AF, Johnson CL. 2000 CDC Growth Charts for the United States: methods and development. *Vital Health Stat.* 2002; 11:1–190.
20. Cook EF, Goldman L. Asymmetric stratification. an outline for an efficient method for controlling confounding in cohort studies. *Am J Epidemiol.* 1988; 127:626–639. [PubMed: 3341363]
21. Ciampi A, Lawless JF, McKinney SM, Singhal K. Regression and recursive partition strategies in the analysis of medical survival data. *J Clin Epidemiol.* 1988; 41:737–748. [PubMed: 3418363]
22. Baracco R, Saadeh S, Valentini R, Kapur G, Jain A, Mattoo TK. Iron deficiency in children with early chronic kidney disease. *Pediatr Nephrol.* 2011; 26:2077–2080. [PubMed: 21710251]

23. Goodnough LT, Nemeth E, Ganz T. Detection, evaluation, and management of iron-restricted erythropoiesis. *Blood*. 2010; 116:4754–4761. [PubMed: 20826717]
24. Fishbane S, Pollack S, Feldman HI, Joffe MM. Iron indices in chronic kidney disease in the National Health and Nutritional Examination Survey 1988–2004. *Clin J Am Soc Nephrol*. 2009; 4:57–61. [PubMed: 18987297]
25. Kalantar-Zadeh K, McAllister CJ, Lehn RS, Liu E, Kopple JD. A low serum iron level is a predictor of poor outcome in hemodialysis patients. *Am J Kidney Dis*. 2004; 43:671–684. [PubMed: 15042544]
26. Sharma AP, McKenna AM, Lepage N, Nieuwenhuys E, Filler G. Relationships among serum iron, inflammation, and body mass index in children. *Adv Pediatr*. 2009; 56:135–144. [PubMed: 19968946]

Fig. 1.
Distribution of iron biomarker values by stage of chronic kidney disease in 304 iron- and erythropoiesis stimulating agent-naïve enrolled in the Chronic Kidney Disease in Children (CKiD) Study

Fig. 2.

Percentile plots of hemoglobin z-score (for age, sex, and race) by Disease Outcomes Quality Initiative (KDOQI) recommended thresholds for ferritin and transferrin saturation (TSAT) in 304 iron supplement- and erythropoiesis stimulating agent-naïve children. *Horizontal lines* of each percentile plot indicate the strata-specific percentiles of hemoglobin z-score indicated on the *left*. Outliers (values < the 2.5 percentile or > the 97.5 percentile) are shown as *solid dots*. The proportion of each hemoglobin z-score distribution corresponding to standardized hemoglobin levels less than the 5th percentile (z-score < -1.64) is shaded in *gray*.

Fig. 3.

Regression tree analysis examining factors predictive of hemoglobin percentile value in 304 iron supplement- and erythropoiesis stimulating agent-naïve children. Each pair of *solid black arrows* represents binary partitioning of the hemoglobin percentile values by the independent variable described in the resulting two boxes. The *p* value between the two boxes quantifies the statistical significance of the difference in hemoglobin percentile values by the partition. *Dashed red lines* represent amalgamations of subgroups (resulting from the partitioning process) with similar hemoglobin percentile distributions. *Ovals at the bottom* of the tree describe the final three unique subgroups of hemoglobin percentile (number of subjects, median [IQR] of hemoglobin percentiles) identified from the analysis. *GFR* glomerular filtration rate (ml/min/1.73 m²), *sFE* serum iron (µg/dl), ferritin units = ng/ml

Table 1

Demographic and clinical characteristics of 304 iron- and erythropoiesis stimulating agent-naïve children enrolled in the Chronic Kidney Disease in Children (CKiD) Study

Characteristic	Median [IQR] or % (n)	GFR, ml/min/1.73 m ²				p value ^a	
	Overall n=304	60 n=88	45–59 n=103	30–44 n=79	<30 n=34		
Age, years	12 [8, 15]	12 [7, 15]	12 [8, 15]	11 [7, 15]	15 [11, 16]		0.11
Male, % (n)	62 % (187)	63 % (55)	58 % (60)	63 % (50)	65 % (22)		0.87
African American race, % (n)	23 % (70)	38 % (33)	17 % (17)	18 % (14)	18 % (6)		0.003
Hispanic ethnicity, % (n)	12 % (37)	6 % (5)	12 % (12)	18 % (14)	18 % (6)		0.07
Glomerular CKD, % (n)	19 % (59)	25 % (22)	16 % (16)	16 % (13)	24 % (8)		0.31
GFR, ml/min/1.73 m ²	50 [40, 63]	71 [66, 83]	52 [48, 56]	39 [35, 43]	25 [22, 28]		
Hemoglobin, g/dl	12.9 [12.1, 13.9]	13.3 [12.4, 14.2]	13.0 [12.3, 13.9]	12.6 [11.9, 13.7]	11.9 [11.1, 12.9]		<0.001
Hemoglobin %ile	31 [6, 74]	54 [27, 86]	30 [9, 74]	21 [3, 67]	3 [<1, 21]		<0.001
Serum iron, 3g/dl	75 [55, 99]	74 [50, 105]	79 [56, 99]	73 [53, 97]	69 [55, 86]		0.54
Serum iron <50 µg/dl	19 % (59)	22 % (19)	17 % (18)	19 % (15)	21 % (7)		0.90
Serum ferritin, ng/ml	33 [22, 50]	27 [20, 47]	33 [19, 50]	33 [22, 48]	47 [27, 82]		0.03
Serum ferritin <12 ng/ml, % (n)	5 % (15)	7 % (6)	6 % (6)	3 % (2)	3 % (1)		0.62
Serum ferritin <100 ng/ml, % (n)	93 % (283)	94 % (83)	95 % (98)	92 % (73)	85 % (29)		0.27
TSAT, %	24 [17, 32]	23 [18, 32]	24 [17, 33]	23 [16, 32]	24 [18, 28]		0.89
TSAT <14 %, % (n)	14 % (42)	16 % (14)	13 % (13)	14 % (11)	12 % (4)		0.93
TSAT <20 %, % (n)	34 % (102)	34 % (30)	35 % (36)	34 % (27)	26 % (9)		0.85
Urine protein (mg/dl)/creatinine (mg/dl), % (n)	0.37 [0.17, 0.99]	0.18 [0.10, 0.39]	0.33 [0.16, 0.73]	0.48 [0.31, 1.43]	1.61 [0.86, 3.21]		<0.001
Significant proteinuria, (0.2–2.0)	55 % (168)	43 % (38)	59 % (61)	66 % (52)	50 % (17)		
Nephrotic proteinuria, 2.0	13 % (39)	2 % (2)	6 % (6)	20 % (16)	44 % (15)		
BMI %ile, % (n)	68 [38, 91]	76 [45, 93]	68 [35, 86]	68 [35, 86]	66 [28, 94]		0.43
Overweight, 85–95 %ile	14 % (44)	19 % (17)	14 % (14)	13 % (10)	9 % (3)		
Obese, >95th %ile	17 % (52)	17 % (15)	13 % (13)	22 % (17)	21 % (7)		

^aComparing four GFR groups: Kruskal–Wallis test for continuous variables; Fisher's exact test for binary/categorical variables

CKD, chronic kidney disease; IQR, interquartile range; GFR, glomerular filtration rate; BMI, body mass index; TSAT, transferrin saturation