Serum biomarkers of iron stores are associated with worse physical health-related quality of life (HRQoL) in non-dialysis dependent chronic kidney disease (NDD-CKD) patients with or without anemia

Murilo Guedes¹,², Daniel Muenz¹, Jarcy Zee¹, Marcelo Barreto Lopes¹, Sandra Waechter³, Bénédicte Stengel⁴, Ziad A. Massy⁴,⁵, Elodie Speyer⁴, Carole Ayav⁴, Fredric Finkelstein⁶, Ricardo Sesso⁷, Ronald L. Pisoni¹, Bruce M. Robinson¹, Roberto Pecoits-Filho¹,²

¹ Arbor Research Collaborative for Health, Ann Arbor, MI, USA
² Pontificia Universidade Catolica do Parana, Curitiba, Brazil
³ Vifor Pharma Ltd, Glattbrugg, Switzerland
⁴ Université Paris-Saclay, Université Versailles Saint-Quentin-en-Yvelines, Université Paris-Sud, Inserm, Équipe Epidémiologie Clinique, CESP, 94807, Villejuif, France
⁵ France, Division of Nephrology, Ambroise Paré University Hospital, APHP, Boulogne-Billancourt/Paris, France
⁶ Yale University, New Haven, CT, USA
⁷ Federal University of Sao Paulo, SP, Brazil

Running Head: ID and patient-reported outcomes in CKD

Correspondence to: Roberto Pecoits-Filho; E-mail: Roberto.Pecoits@ArborResearch.org

© The Author(s) 2021. Published by Oxford University Press on behalf of ERA-EDTA.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
ABSTRACT

Background. Iron deficiency (ID) is a common condition in non-dialysis dependent chronic kidney disease (NDD-CKD) patients that is associated with poorer clinical outcomes. However, the effect of ID on health-related quality of life (HRQoL) in this population is unknown. We analyzed data from a multinational cohort of NDD-CKD stage 3 to 5 patients to test the association between transferrin saturation index (TSAT) and ferritin with HRQoL.

Methods. Patients from Brazil (N=205), France (N=2015), and the US (N=293) in the CKD Outcomes and Practice Patterns Study (CKDopps, 2013 to 2019) were included. We evaluated the association of TSAT and ferritin (and functional and absolute ID, defined as TSAT ≤ 20% and ferritin >300 or <50 ng/mL, respectively) on pre-specified HRQoL measures, including KDQOL-36 PCS and MCS as the primary outcomes. Models were adjusted for confounders including hemoglobin (Hgb).

Results. TSAT ≤15% and both ferritin <50 ng/mL and ≥300 ng/mL were associated with worse PCS scores, but not with MCS. Patients with composite TSAT ≤20% and ferritin <50 or ≥300 ng/mL had lower functional status and worse PCS than those with TSAT of 20%-30% and ferritin 50-299 ng/mL. Patients with lower TSAT were less likely to perform intense physical activity. Adjustment for Hgb only slightly attenuated the observed effects.

Conclusions. Low TSAT levels, as well as both low TSAT with lower ferritin and low TSAT with high ferritin, are associated with worse physical HRQoL in NDD-CKD patients, even after accounting for Hgb level. Interventional studies of iron therapy on HRQoL among NDD-CKD individuals are needed to confirm these findings.

Keywords: chronic kidney disease, health-related quality of life, iron deficiency
KEY LEARNING POINTS

What is already known about this subject?

Iron plays a crucial role in biological functions beyond erythropoiesis, including energy cell metabolism. ID correction improves clinical and patient-reported outcomes in patients with chronic diseases, such as heart failure.

What this study adds?

In NDD-CKD patients, clinical trials in ID have been limited to erythropoetic-focused goals, lacking patient-reported outcomes (PRO); we sought to explore the associations between iron stores and PROs among ND-CKD patients with or without anemia.

Our description of the associations between ID and physical aspects of HRQoL independent of hemoglobin levels supports the hypothesis that a decrease in iron availability in the body results not only in anemia, but also in a disarrangement of energy metabolism.

What impact this may have on practice or policy?

Our results support the need for randomized controlled trials for a shift in the current paradigm, in which ID is screened and managed only in anemic patients with a focus on promoting erythropoiesis, to a broader approach in which the management of ID could improve quality of life. New randomized controlled trials may further test this hypothesis and help foster a more patient-centered approach to ID management in CKD care.
INTRODUCTION

In biological systems, iron exerts essential functions and can potentially result in cell toxicity, particularly in the context of increased intra-cellular iron content resulting in oxidative injury, which explains the complex regulation of iron metabolism in human physiology. On the other hand, a decrease in iron availability in the body results not only in iron deficiency anemia, but also in a disarrangement of the energy metabolism, particularly in muscle cells, given that iron is essential for the synthesis of major proteins in a wide range of intracellular pathways.

A precise diagnosis of iron deficiency (ID) can be achieved by the confirmation of reduced iron content in the bone marrow, although the clinical use of this evaluation is not practical. Albeit far from ideal, the most commonly used parameters to assess iron status in clinical practice are the transferrin saturation (TSAT) and ferritin. Based on these biochemical parameters, there are two main ID subtypes: absolute ID, loosely defined as low TSAT and low ferritin levels, and functional ID – high ferritin combined with low TSAT. While the former represents the most common subtype in the general population, the latter is commonly found in chronic inflammatory states, such as heart failure (HF) and chronic kidney disease (CKD). In fact, functional ID results in part from increased hepcidin levels, which reduce iron release from the reticuloendothelial system, thereby restricting iron availability for metabolic functions.

ID is a common finding in non-dialysis dependent chronic kidney disease (NDD-CKD), occurring in up to 50% of patients with anemia. Current CKD guidelines recommend that screening for ID should be done mainly in the context of anemia. Previous studies have shown that even in this restricted strategy of testing for iron parameters, patients are often left under-evaluated and under-treated for ID.
The clinical effects of ID on muscle metabolism and function are well described in chronic conditions having a similar pathophysiological basis for ID, such as HF. In HF patients, ID, independently from anemia, has been associated with worse functional and patient-reported outcomes in observational studies \(^{10,11}\). Randomized controlled studies (RCTs) have confirmed the benefits of ID treatment in HF, regardless of anemia status, with improvements ranging from patient-reported to clinical outcomes \(^{12,13}\). In NDD-CKD patients, ID, both functional and absolute, has been associated with worse clinical outcomes in observational studies \(^{14,15}\); RCTs, however, have primarily focused on the erythropoietic effects of iron replacement therapy \(^{16,17}\).

In NDD-CKD, the extent to which serum biomarkers of ID, as assessed by TSAT and ferritin, are associated with worse HRQoL, and particularly independent from anemic states, has not been previously investigated. We therefore designed an analysis using data from CKDopps \(^{18}\), an ongoing international prospective cohort study of adult NDD-CKD patients, to address the following hypotheses: i) low TSAT, including its combinations with ferritin (i.e., high and low ferritin), is associated with worse HRQoL, particularly in physical domains, among NDD-CKD persons; and ii) the association between serum biomarkers of iron stores and HRQoL is not mediated or modified by hemoglobin (Hgb) levels.

**MATERIALS AND METHODS**

**Patient Sample**

CKDopps is an ongoing prospective cohort study of Stage 3-5 NDD-CKD patients treated in nephrologist-led CKD clinics in Brazil, France, Germany, Japan, and the United States (US). CKDopps sites were randomly selected from CKD clinics after stratification by region. CKDopps
study design, details, and objectives have previously been published. CKDopps was approved by national and/or local ethics committees and patient consent obtained as required by local ethics regulations.

Our analyses included French, Brazilian, and US patients (2013-2019). German patients were not administered the HRQoL questionnaires, while data from Japan were unavailable at the time of analysis. The analysis cohort is comprised primarily (80%) of French patients due to higher recruitment targets in CKDopps compared to other countries, greater patient completion of the HRQoL questionnaire, and greater availability of TSAT and ferritin measurements due to a France-specific protocol requirement for collection.

**Exposure Definition**

The exposures were defined as the closest single TSAT/ferritin measurement reported within 180 days before collection of the HRQoL data. When available, TSAT and ferritin labs were required to be collected on the same day. We used multiple imputation if one of these two was not reported, assuming they were missing at random; all patients were required to have at least one of the two labs for inclusion in our analysis. Median days from exposure to HRQoL data was 24 (interquartile range [IQR], 13-49).

We treated TSAT and ferritin as exposures separately for the primary analyses. TSAT was categorized as ≤15%, >15%-20%, >20%-30% (reference group), >30%-50%, and >50%, with ferritin categorized as <50 ng/mL, 50-99 ng/mL, 100-299 ng/mL (reference group), and ≥300 ng/mL. We also considered joint categories of TSAT and ferritin, defining ferritin as <50 ng/mL, 50-299 ng/mL, and ≥300 ng/mL, while TSAT categories were ≤20%, >20%-30%, >30%-50%, and
>50%; the joint categories used wider ranges than in the primary analyses to maintain sufficient sample size within each category.

**Outcomes**

*Primary Outcome*

**KDQOL-36 PCS and MCS**

The KDQOL-36 questionnaire combines both general HRQoL measures and kidney-specific domains.\textsuperscript{19,20} Items were summarized to yield physical component summary (PCS) and mental component summary (MCS) scores, along with burden, symptoms, and effects of kidney disease.

*Secondary Outcomes*

**Self-Reported Physical Activity**

Self-reported physical activity, categorized as low, moderate, or intense, was assessed by the Global Physical Activity Questionnaire (GPAQ), whose validation and reliability for the general population has been previously evaluated\textsuperscript{21}. For this analysis, we created a binary outcome combining the low and moderate categories\textsuperscript{22}. As the GPAQ was collected only in the French cohort, our analysis of physical activity was restricted to this cohort.

**Depression**

The short form of the Center for Epidemiologic Studies Depression Scale (CES-D) consists of 10 items for depression screening and has been validated in the general population\textsuperscript{23}. We
followed the standard procedure of scoring each item from 0-3 and summing to create a single continuous score from 0-30, with higher scores indicating greater symptoms of depression ²³.

**Functional status**

Functional status was defined by the Activities of Daily Living (ADL) Katz instrument and the Instrumental Activities of Daily Living (IADL) Lawton-Brody scale; both were previously validated in the general population ²⁴,²⁵. In brief, these instruments assess the respondent’s independency for performing a set of activities (e.g., eating, getting dressed, doing laundry) and are strongly correlated with poor prognosis and more health-care system use both in the general population and in end-stage renal disease (ESRD) patients ²⁶. For this analysis, we combined the ADL and IADL to create a single binary outcome indicating highest functional status, i.e., no help needed to complete the daily activities.

**Exploratory Outcomes**

**SF-12**

We calculated scores for the eight MCS and PCS SF-12 subdomains: emotional role, emotional well-being, energy, general health, pain, physical function, physical role, and social functioning.

We performed an exploratory analysis of subdomains of SF-12.

**Statistical Analyses**

For all outcomes, both continuous and binary, linear mixed models were built with a random clinic intercept and fixed effects for multiple confounders. For continuous outcomes, the
models estimated the mean differences in the outcome scores across exposure categories. For binary outcomes, the models estimated the differences in probabilities of the outcome across exposure categories. Models were adjusted for country, age, sex, Black race, body mass index (BMI), current smoker, estimated glomerular filtration rate (eGFR), albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score (a score from 0-3, indicating the presence of coronary artery disease, cerebrovascular disease, and/or peripheral vascular disease), congestive heart failure, other cardiovascular disease, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene, and ESA prescription.

Two sets of models, one with and one without adjustment for hemoglobin level, were constructed to assess whether hemoglobin could be a potential mediator of the effect of ID on HRQoL. Hemoglobin values reported on the same day as iron stores were considered. Models with TSAT exposure were also adjusted for ferritin, while models with ferritin exposure were adjusted for TSAT.

We performed subgroup analyses for all outcomes stratified according to CKD stage (stage 3 vs. 4 and 5), iron treatment (any intravenous or oral iron vs. none in the six months before the exposure), and anemia status (defined as Hb <13.5 g/dL in men and 12 g/dL in women).

Multiple imputation, implemented by IVEware, was used in all analyses to impute missing exposure and covariate values. Missingness was 13% for TSAT and 4% for ferritin; per our inclusion criteria, all patients had at least one of these measurements. Missingness was <5% for all other covariates except albuminuria (11%) and serum albumin (17%). Twenty complete data sets were imputed, all analyses were performed with each imputed data set, and results were combined using Rubin’s rules.
All analyses used SAS software, version 9.4 (SAS institute, Cary, NC). Confidence intervals (CIs) are reported with 95% confidence level.

RESULTS

A total of 2513 NDD-CKD patients from France (n=2015, 80%), Brazil (n=205, 8%) and the US (n=293, 12%) were eligible for inclusion in this analysis (Supplemental Figure 1). Patient characteristics are shown in Table 1.

Patient distribution across TSAT categories of ≤15%, >15%-20%, >20%-30%, >30%-50%, and >50% was 14%, 19%, 42%, 23%, and 2% of patients, respectively. Patients in the higher TSAT categories were younger, were more likely to be male, had lower BMI, higher serum ferritin levels, and lower prevalence of comorbidities (Table 1). Moreover, patients in the highest TSAT categories were more likely to receive ESAs, whereas iron therapy was more likely prescribed for those who had higher and lower TSAT levels. Mean eGFR was similar across TSAT groups.

Regarding ferritin, there were 13%, 23%, 46%, and 18% persons distributed in ferritin categories of <50 ng/mL, 50-99 ng/mL, 100-299 ng/mL, and ≥300 ng/mL. Differences in patient characteristics across ferritin categories were less pronounced compared to those across TSAT categories (Table 1). As defined in this study, the sample proportion of patients with combinations of low TSAT with low ferritin and low TSAT with high ferritin were 8% and 4%, respectively. Patient characteristics across TSAT and ferritin combinations are depicted in Supplemental Table 1.
Mean PCS and MCS scores for this population were 41 and 45, respectively. Means for PCS and the physical subdomains of the KDQOL-36 tended to be progressively lower at lower TSAT levels. In contrast, MCS and mental component subdomains varied much less across TSAT levels (Table 2). Relatively small differences were seen in mean PCS and MCS scores across ferritin categories. Descriptives for other patient-reported outcomes (PROs) are shown in Table 2.

Patients with TSAT ≤15% had -1.8 (95% CI: -3.1, -0.5) adjusted mean difference in PCS and -0.7 (95% CI: -2.2, 0.7) mean difference in MCS compared to patients with TSAT >20%-30% (Figure 1). Patients with ferritin ≥300 ng/mL had -1.3 (95% CI: -2.4, -0.3) mean difference for PCS and 0.2 (95% CI: -1.0, 1.5) for MCS compared to the reference ferritin group of 100-299 ng/mL, while those with ferritin <50 ng/mL had -1.1 (95% CI: -2.4, 0.1) mean difference for PCS and 0.0 (95% CI: -1.4, 1.4) for MCS (Figure 1). Adjustment for hemoglobin only slightly attenuated the observed effect sizes. Furthermore, similar results were seen in subgroup analyses stratified according to CKD stage, including for patients with stage 3 vs. stage 4 and 5 CKD, for those prescribed vs. not prescribed iron treatment, and for those with hemoglobin ≥11.5 vs. <11.5 g/dL.

We further investigated the relationship of outcomes with joint categories of TSAT and ferritin. Individuals with TSAT ≤20% and ferritin <50 ng/dL had -2.3 lower PCS (95% CI: -3.9, -0.8) compared to those having TSAT >20%-30% and ferritin 50-299 ng/mL (reference group). Moreover, those with TSAT ≤20% and ferritin ≥300 ng/mL had even lower PCS scores (-3.6 [95% CI: -5.7, -1.6]). Consistently, adjustment for hemoglobin resulted in only a small or no attenuation in the effect sizes for both PCS and MCS (Figure 2).
Analyses of the KDQOL-36 kidney domains showed that, compared to those with TSAT >20%-30%, patients with TSAT <15% had worse HRQoL for effects of kidney disease, while mean differences for symptoms and burden were smaller between groups (Supplemental Figure 2). Regarding ferritin, only small differences were seen in the mean values for these domains (Supplemental Figure 2). Investigation of the subdomains of PCS and MCS revealed modest associations for physical-related HRQoL subdomains in relationship to TSAT and ferritin levels (Supplemental Figure 3). For TSAT, the subdomain scores of general health, pain, physical function, and energy were consistently lower for persons with TSAT <20% (Supplemental Figure 3). For ferritin, overall, both higher and lower levels were associated with worse subdomain scores, particularly for physical function, physical role, and pain (Supplemental Figure 4). Consistent with findings for mental domains, there was little difference in mean CESD-10 levels across TSAT and ferritin categories (Supplemental Figure 5).

For functional status, the difference in probability of having at least one impairment was similar across TSAT categories, while patients with ferritin >300 ng/mL had a higher risk of functional dependency (Figure 3). Considering the joint exposure of TSAT and ferritin, patients with TSAT <20% and ferritin >300 ng/mL had a 15-percentage point (95% CI: 4.6, 25.3) higher probability of having impairment compared to patients with TSAT >20%-30% and ferritin 50-299 ng/mL. Effect sizes were not materially changed by adjustment for Hgb (Supplemental Table 2). For self-reported physical activity, patients with TSAT <20% had a lower probability of routinely performing intense physical activity, compared to those with TSAT >20%-30%. For ferritin, there was little difference in the probability of self-reported physical activity (Figure 3).
DISCUSSION

In this observational study using data derived from a multinational study in patients with moderate to advanced CKD, isolate low TSAT and combinations of low TSAT with high and low ferritin were associated with worse patient-reported outcomes, particularly for physical domains of HRQoL. Importantly, these associations were not materially affected by the adjustment for Hgb levels and were consistent among subgroups of anemic vs. non-anemic individuals, suggesting that the observed effect (whether biologically causal or not) is direct rather than mediated through Hgb. To the best of the authors’ knowledge, this is the first evidence suggesting that serum biomarkers of iron stores are associated with worse PROs among individuals with NDD-CKD.

In our study, we assumed patients with TSAT lower than 20% and ferritin greater than 300 ng/mL would be representative of functional ID (iron restriction), while TSAT lower than 20% and ferritin lower than 50 ng/mL as the absolute ID subtype (iron depletion); we considered the fact that the exact cut-off for such definitions have been largely debated and have demonstrated great variability across medical specialties. To guide our choice of cut-offs in the spectrum of ID phenotypes, we used ranges defined for the HF population, in which well-designed RCTs have shown that iron supplementation for patients with iron depletion improves cardiovascular outcomes. Particularly, we assumed patients with TSAT ≤ 20% and ferritin < 50 ng/mL are more likely iron depleted, considering the mean values of those parameters in the aforementioned HF trials. For the upper range of ferritin, we assumed patients with ferritin greater than 300 ng/mL, which is the maximum range for inclusion in these trials, would most likely reflect iron restriction, rather than depletion. Our findings show that patients with
lower TSAT were more likely to have more comorbidities, including diabetes and cardiovascular diseases, and were more likely to be prescribed iron treatment, mostly oral formulations. Consistent with previous CKDopps data, eGFR was similar across TSAT categories, while higher ferritin was correlated with lower eGFR, which may be a result of the progressive inflammatory phenotype observed in more advanced CKD. This is also consistent with the observation that, in the present analysis, functional iron deficiency was higher among those with more advanced CKD, which confirms findings in previous cohort studies.

In an analysis of a previous cohort study, ID has been associated with higher mortality risk among NDD-CKD patients. Particularly, patients with functional ID tended to have a slightly higher risk of mortality and cardiovascular events compared to those with absolute ID. Although this could be driven by underlying comorbidities contributing to the release of hepcidin and independently causing worse outcomes, it remains possible that in such iron-restricted states due to inflammation, tissue iron deficiency as a result of a mismatch between iron supply and demand may impact cell function, in a similar condition as in absolute iron deficiency. At a cellular level in non-erythropoietic tissues, the impact of an unmet need of iron in such hepcidin-induced restriction remains to be studied. In spite of these considerations, whether treatment of ID, either with or without anemia, improves clinical outcomes among NDD-CKD patients remains unknown. In other populations with chronic diseases associated with inflammatory phenotypes such as HF, treatment of ID improves physiological surrogates, such as ejection fraction and functional measures, as well as patient-reported outcomes and cardiovascular events independently from anemia status, as demonstrated by clinical trials.
such patients, independently of its erythropoietic effects, are highlighted by the concept of tissue iron deficiency, given that iron is essential for the synthesis of many enzymes for cell energy metabolism, including mitochondrial electron transport chain components. Further studies are needed to clarify the clinical relevance of this concept.

The main finding of our study was the consistent association between serum biomarkers of iron stores and PROs, particularly those related to physical domains. Lower TSAT was associated with worse PCS, with small effect estimates sustained even after adjustment for hemoglobin. Among subdomains of the KDQOL-36, lower TSAT was associated with worse physical HRQoL, specifically for general health, physical role, physical function, and energy, with mean differences as high as 5 points, which could translate into clinically meaningful differences (MID), according to studies in patients with CKD anemia and consistent with previous DOPPS analyses. Accordingly, patients with lower TSAT were less likely to perform higher intensity physical activity captured by the GPAQ, which further reinforces the potential impact of tissue iron deficiency on muscle and physical function, as has been extensively studied in heart failure patients. Compared to TSAT, ferritin displayed a more J-shaped pattern of associations with physical HRQoL, with both higher and lower ferritin levels associated with worse PROs. Importantly, patients with higher ferritin may have restricted iron availability via hepcidin-mediated pathways, characterizing functional ID. In fact, we found that the subgroup of patients with high ferritin and low TSAT had generally worse physical PROs compared to absolute ID persons. Notably, higher ferritin levels were strongly associated with higher risk of having daily living activities impairment. Our analyses showed that this effect was driven primarily by those patients who also had a TSAT lower than 20%, with a 15% higher probability
of worse functionality in this subgroup compared to those with TSAT between 20%-30% and ferritin 50-299 ng/mL. Consistently, these effect estimates remained after adjustment for hemoglobin, which suggests that, potentially, functional ID has a potential role in worse functional status, which may be the long-term clinical consequence of physical dysfunction and restricted physical activity in the course of CKD. Whether new approaches tackling functional ID could slow the progression of physical impairment to restricted functionality in NDD-CKD is currently unknown.

On the other hand, our effect estimates for mental domains were generally neutral for TSAT, ferritin, and the joint TSAT/ferritin exposures. Self-reported depressive symptoms were similar across exposure categories, as well as mental sub-domains in the KDQOL-36. In particular, the only MCS subdomain that showed associations with iron parameters was energy, which has been shown to be particularly associated with CKD anemia. These results further reinforce the hypothesis that iron deficiency has a primary impact on physical function, probably through tissue iron deficiency mainly affecting energy metabolism in muscle tissues, which could explain major benefits of iron treatment in physical function in patients with comorbidities such as heart failure.

Our subgroup analyses did not suggest any effect modification on these outcomes by CKD stage, anemia, or iron treatment. Generally, we defined our approach following a set of assumptions about the causal structure of iron status, hemoglobin, and PROs. We assumed hemoglobin would be a potential mediator of the associations between iron parameters and PROs. Therefore, in our study design, we did not include hemoglobin data preceding the measurement of TSAT/ferritin. Also, we defined at least two sets of fully adjusted models for
confounders, one including hemoglobin and another without it, providing comparisons of the
effect estimates after adjusting for the potential mediator variable. Under these assumptions,
our results suggest that the associations we report are directly driven from ID. Interventional
studies are needed to confirm these assumptions.

Our study has limitations. Due to the observational nature of our study, we cannot rule out
residual confounding for the associations we provided. Moreover, due to exclusion of patients
who did not complete PRO questionnaires in our sample, we cannot rule out selection bias in
our analysis, which may affect external validity of our estimates. The results of our exploratory
analysis of the SF-12 subscales should be interpreted cautiously, given that several subdomains
are defined by single items, which can limit the validity of the estimates. Although our results
for these subdomains are purely exploratory, new studies are needed to confirm our findings.
Although most of our effect estimates for the KDQOL-36 scale could be considered to be only
modest under traditional ranges for MID, it’s important to consider that methods for
determining MIDs can vary according to the disease state, potential intervention, and patient
population. Potential improvements in PROs, even modest in magnitude, achieved by low
cost or highly available interventions could translate into important populational benefits. The
modest associations between KDQOL-36 scales and serum markers of iron stores shown here
could be used to pursue and plan clinical trials evaluating the efficacy of iron supplementation
on PROs. Moreover, we decided not to define our exposures strictly according to guideline-
defined cut-offs for ID, as these are generally arbitrary and vary considerably both within
nephrology and across different specialties. Therefore, the sample prevalence of patients
with both low TSAT with low ferritin and low TSAT and high ferritin were relatively low (8% and
4%, respectively). In addition, whether current guideline-based targets for iron-deficiency anemia management are also appropriate for correcting ID in isolation is still uncertain. Our sample was mainly composed of patients from France, which may limit the external validity of our findings; also, the analysis of the physical activity component was restricted to only the French cohort. Finally, we used a single measurement of serum biomarkers of iron stores to define the exposure in our analysis, and although we recognize the variation in those biomarkers and the impact of treatment over time, the infrequent monitoring of iron stores and the intense variability in treatment patterns observed in a previous analysis of our cohort makes the longitudinal approach to the analysis unfeasible. On the other hand, our results are consistent with robust evidence from the HF population, which is supported by well-designed observational studies and also RCTs. Finally, our findings are also consistent across distinct PRO instruments with similar dimensions, reflecting a broad impact of ID on physical aspects of HRQoL.

Our study provides new insights for potential strategies to improve PROs in NDD-CKD care. Iron treatments are widely available and safe, and ID is highly prevalent among patients with CKD. It will be important to design intervention studies to analyse the hypothesis raised by our observation (and supported by trials in HF) that treating ID, even in the absence of anemia, may improve the perception of physical function, the capacity to execute physical activities and, importantly, the ability to perform daily activities.

In conclusion, low TSAT, as well as both low and high ferritin levels, are associated with worse physical HRQoL in NDD-CKD patients, even after adjustment or stratification by Hgb level. Randomized controlled studies addressing the potential impact of iron replacement therapies
on the HRQoL of NDD-CKD individuals with and without anemia are needed to confirm the associations observed in this cohort.

Supplementary Material Table of Contents

Supplemental Table 1. Patient characteristics by TSAT and ferritin cross-classification

Supplemental Table 2. Mean differences in all outcomes by TSAT and ferritin levels, with and without adjustment for Hgb

Supplemental Figure 1. Flow-chart diagram for inclusion process.

Supplemental Figure 2. Mean differences in effects, symptoms, and burden of kidney disease by TSAT and ferritin levels, with and without adjustment for Hgb. Models were adjusted for country, age, sex, Black race, body mass index, current smoker, estimated glomerular filtration rate, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive heart failure, other cardiovascular disease, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene, and erythropoietin stimulating agents. Additionally, models with TSAT exposure were adjusted for ferritin, and vice versa.

Supplemental Figure 3. Mean differences in subdomains of PCS/MCS by TSAT level, with and without adjustment for Hgb. Models were adjusted for country, age, sex, Black race, body mass index, current smoker, estimated glomerular filtration rate, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive heart failure, other cardiovascular disease, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene, erythropoietin stimulating agents, and ferritin.

Supplemental Figure 4. Mean differences in subdomains of PCS/MCS by Ferritin level, with and without adjustment for Hgb. Models were adjusted for country, age, sex, Black race, body mass index, current smoker, estimated glomerular filtration rate, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive heart failure, other cardiovascular disease, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene, erythropoietin stimulating agents, and TSAT.

Supplemental Figure 5. Mean differences in CES-D 10 score by TSAT and ferritin levels, with and without adjustment for Hgb. The CES-D 10 score ranges from 0 to 30. Higher scores are worse. Models were adjusted for country, age, sex, Black race, body mass index, current smoker, estimated glomerular filtration rate, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive heart failure, other cardiovascular disease, gastrointestinal bleeding, lung disease, cancer,
ulcers/gangrene, and erythropoietin stimulating agents. Additionally, models with TSAT exposure were adjusted for ferritin, and vice versa.

ACKNOWLEDGEMENTS

*CKDopps Investigators

CKDopps Steering Committee and Country Investigators: Antonio Lopes, Roberto Pecoits-Filho (Brazil); Christian Combe, Christian Jacquelinet, Ziad Massy, Benedicte Stengel (France); Johannes Duttlinger, Danilo Fliser, Gerhard Lonnemann, Helmut Reichel (Germany); Takashi Wada, Kunihiro Yamagata (Japan); Ron Pisoni, Bruce Robinson (United States).

Additional CKDopps Research Group: Viviane Calice da Silva, Ricardo Sesso (Brazil); Elodie Speyer (France); Koichi Asahi, Junichi Hoshino, Ichiei Narita (Japan); Rachel Perlman, Friedrich Port, Nidhi Sukul, Michelle Wong, Eric Young, Jarcy Zee (United States).

In France, CKDopps is part of the CKD-REIN cohort, which is funded by the Agence Nationale de la Recherche through the 2010 «Cohortes-Investissements d’Avenir » program and by the 2010 national Programme Hospitalier de Recherche Clinique. CKD-REIN is also supported through a public-private partnership with Amgen, Fresenius Medical Care, and GlaxoSmithKline (GSK) since 2012, Baxter and Merck Sharp & Dohme-Chibret (MSD France), from 2012 to 2017, Lilly France since 2013, and Otsuka Pharmaceutical since 2015, Vifor Fresenius since 2017, and Sanofi-Genzyme from 2012 to 2015.

In the United States and Brazil support for the CKDopps Coordinating Center has been provided by Keryx.

Jennifer McCready-Maynes, and employee of Arbor Research Collaborative for Health, provided editorial assistance.

CONFLICT OF INTEREST STATEMENT

Murilo Guedes, Daniel Muenz, Jarcy Zee, Marcelo Barreto Lopes, Ronald L. Pisoni, Bruce M. Robinson, and Roberto Pecoits-Filho are employees of Arbor Research Collaborative for Health, which administers the DOPPS programs. Global support for the ongoing DOPPS Programs is provided without restriction on publications by a variety of funders. For details see https://www.dopps.org/AboutUs/Support.aspx. This manuscript was directly supported by Vifor Pharma. In addition, Dr Robinson has received consultancy fees or travel reimbursement.
in the last three years from AstraZeneca, GlaxoSmithKline, and Kyowa Kirin Co., all paid directly to his institution of employment. Dr. Pecoits-Filho reports grants from Fresenius Medical Care, non-financial support from AstraZeneca, non-financial support from Novo Nordisk, non-financial support from Akebia, personal fees from Retrophin, outside the submitted work.

Fredric Finkelstein, Ricardo Sesso, and Carole Ayav have nothing to disclose.

Ziad Massy reports grants and other from Amgen, grants and other from Sanofi-Genzyme, grants from French Government, grants from MSD, grants from GSK, grants from Lilly, grants from FMC, grants and other from Baxter, grants from Outsuka, other from Daichi, other from Astellas, outside the submitted work.

Elodie Speyer reports grants from Vifor Fresenius, grants from Amgen, grants from Otsuka, grants from Fresenius, grants from GSK, grants from AstraZeneca, during the conduct of the study.

Bénédicte Stengel reports grants from Vifor Fresenius, grants from Amgen, grants from Otsuka, grants from Fresenius, grants from GSK, grants from AstraZeneca, during the conduct of the study.

Dr. Waechter reports and is an employee of Vifor Fresenius Medical Care.

AUTHORS’ CONTRIBUTIONS

a. Conceived and/or designed the work that led to the submission, acquired data, and/or played an important role in interpreting the results: RLP, MG, SW, DM, RPF, BMR, ZM, CA, JZ, RS, ES, BS

b. Drafted or revised the manuscript: RLP, MG, SW, DM, RPF, BMR, ZM, JZ, MBL, RS, ES, FF

c. Approved the final version: RLP, MG, SW, DM, RPF, BMR, CA, JZ, MBL, RS, ES, BS, FF

Each author contributed important intellectual content during manuscript drafting or revision, accepts personal accountability for the author’s own contributions, and agrees to ensure that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

The results presented in this paper have not been published previously in whole or part, except in abstract form.
FUNDING

Global support for the ongoing DOPPS Programs is provided without restriction on publications by a variety of funders. For details see https://www.dopps.org/AboutUs/Support.aspx. This manuscript was directly supported by Vifor.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from Arbor Research Collaborative for Health, but restrictions apply to the availability of these data which were used for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Arbor Research Collaborative for Health.
REFERENCES

1. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian iron metabolism. Cell 2010;142:24-38.
2. Beard JL. Iron Biology in Immune Function, Muscle Metabolism and Neuronal Functioning. The Journal of Nutrition 2001;131:568S-80S.
3. Group KDIGOKAW. KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease. Kidney International Supplements 2012;2:279-335.
4. De Franceschi L, Iolascon A, Taher A, Cappellini MD. Clinical management of iron deficiency anemia in adults: Systemic review on advances in diagnosis and treatment. Eur J Intern Med 2017;42:16-23.
5. Panwar B, Gutierrez OM. Disorders of Iron Metabolism and Anemia in Chronic Kidney Disease. Semin Nephrol 2016;36:252-61.
6. Batchelor EK, Kapitsinou P, Pergola PE, Kovesdy CP, Jalal DI. Iron Deficiency in Chronic Kidney Disease: Updates on Pathophysiology, Diagnosis, and Treatment. J Am Soc Nephrol 2020;31:456-68.
7. Fishbane S, Spinowitz B. Update on Anemia in ESRD and Earlier Stages of CKD: Core Curriculum 2018. Am J Kidney Dis 2018;71:423-35.
8. Lawler EV, Gagnon DR, Fink J, et al. Initiation of anaemia management in patients with chronic kidney disease not on dialysis in the Veterans Health Administration. Nephrol Dial Transplant 2010;25:2237-44.
9. Wong MMY, Tu C, Li Y, Perlman RL, et al. Anemia and iron deficiency among chronic kidney disease Stages 3-5ND patients in the Chronic Kidney Disease Outcomes and Practice Patterns Study: often unmeasured, rarely treated. Clin Kidney J. 2018 Aug 3;11(4):631-624.
10. Martens P, Nijst P, Verbrugge FH, Smeets K, Dupont M, Mullens W. Impact of iron deficiency on exercise capacity and outcome in heart failure with reduced, mid-range and preserved ejection fraction. Acta Cardiol 2018;73:115-23.
11. Jankowska EA, Rozentryt P, Witkowska A, et al. Iron deficiency predicts impaired exercise capacity in patients with systolic chronic heart failure. J Card Fail 2011;17:899-906.
12. Anker SD, Comin Colet J, Filippatos G, et al. Ferric carboxymaltose in patients with heart failure and iron deficiency. N Engl J Med 2009;361:2436-48.
13. Anker SD, Kirwan BA, van Veldhuisen DJ, et al. Effects of ferric carboxymaltose on hospitalisations and mortality rates in iron-deficient heart failure patients: an individual patient data meta-analysis. Eur J Heart Fail 2018;20:125-33.
14. Awan AA, Walther CP, Richardson PA, Shah M, Winkelmayr WC, Navaneethan SD. Prevalence, correlates and outcomes of absolute and functional iron deficiency anemia in nondialysis-dependent chronic kidney disease. Nephrol Dial Transplant 2019.
15. Cho ME, Hansen JL, Peters CB, Cheung AK, Greene T, Sauer BC. An increased mortality risk is associated with abnormal iron status in diabetic and non-diabetic Veterans with predialysis chronic kidney disease. Kid Interv 2019;96:750-60.
16. Macdougall IC, Bock AH, Carrera F, et al. FIND-CKD: a randomized trial of intravenous ferric carboxymaltose versus oral iron in patients with chronic kidney disease and iron deficiency anaemia. Nephrol Dial Transplant 2014;29:2075-84.
17. Shepshelovich D, Rozen-Zvi B, Avni T, Gafter U, Gafter-Gvili A. Intravenous Versus Oral Iron Supplementation for the Treatment of Anemia in CKD: An Updated Systematic Review and Meta-analysis. Am J Kidney Dis 2016;68:677-90.
18. Mariani L, Stengel B, Combe C, et al. The CKD Outcomes and Practice Patterns Study (CKDopps): Rationale and Methods. Am J Kidney Dis 2016;68:402-13.
19. Hays RD, Kallich JD, Mapes DL, Coons SJ, Carter WB. Development of the kidney disease quality of life (KDQOL) instrument. Qual Life Res 1994;3:329-38.

20. Ware J, Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care 1996;34:220-33.

21. Bull FC, Maslin TS, Armstrong T. Global physical activity questionnaire (GPAQ): nine country reliability and validity study. J Phys Act Health 2009;6:790-804.

22. Organization WH. Global Physical Activity Questionnaire, (GPAQ) Analysis Guide. 2020.

23. Andresen EM, Malmgren JA, Carter WB, Patrick DL. Screening for depression in well older adults: evaluation of a short form of the CES-D (Center for Epidemiologic Studies Depression Scale). Am J Prev Med 1994;10:77-84.

24. Katz S, Downs TD, Cash HR, Grotz RC. Progress in development of the index of ADL. Gerontologist 1970;10:20-30.

25. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist 1969;9:179-86.

26. Jassal SV, Karaboyas A, Comment LA, et al. Functional Dependence and Mortality in the International Dialysis Outcomes and Practice Patterns Study (DOPPS). Am J Kidney Dis 2016;67:283-92.

27. Raghunathan TE, Solenberger PW, Van Hoewyk J. IVEware: Imputation and variance estimation software. Survey Methodology Program, Survey Research Center, Institute for Social Research, University of Michigan; 2002.

28. Rubin DB. Multiple imputation for nonresponse in surveys. Hoboken, NJ. John Wiley & Sons; 2004.

29. Cappellini MD, Comin-Colet J, de Francisco A, et al. Iron deficiency across chronic inflammatory conditions: International expert opinion on definition, diagnosis, and management. Am J Hematol 2017;92:1068-78.

30. Ponikowski P, Kirwan BA, Anker SD, et al. Ferric carboxymaltose for iron deficiency at discharge after acute heart failure: a multicentre, double-blind, randomised, controlled trial. Lancet. 2020 Dec 12;396(10266):1895-1904.

31. Melenovsky V, Petrak J, Mracek T, et al. Myocardial iron content and mitochondrial function in human heart failure: a direct tissue analysis. Eur J Heart Fail. 2017 Apr;19(4):522-530.

32. Bekfani T, Pellecori P, Morris D, et al. Iron deficiency in patients with heart failure with preserved ejection fraction and its association with reduced exercise capacity, muscle strength and quality of life. Clin Res Cardiol 2019;108:203-11.

33. Lewis GD, Malhotra R, Hernandez AF, et al. Effect of Oral Iron Repletion on Exercise Capacity in Patients With Heart Failure With Reduced Ejection Fraction and Iron Deficiency: The IRONOUT HF Randomized Clinical Trial. JAMA 2017;317:1958-66.

34. Paul BT, Manz DH, Torti FM, Torti SV. Mitochondria and Iron: current questions. Expert Rev Hematol 2017;10:65-79.

35. Finkelstein FO, van Nooten F, Wiklund I, Trundell D, Cella D. Measurement properties of the Short Form-36 (SF-36) and the Functional Assessment of Cancer Therapy - Anemia (FACT-An) in patients with anemia associated with chronic kidney disease. Health Quality Life Outcomes 2018;16:111.

36. Mapes DL, Bragg-Gresham JL, Bommer J, Fukuhara S, McKevitt P, Wikström B, Lopes AA. Health-related quality of life in the Dialysis Outcomes and Practice Patterns Study (DOPPS). Am J Kidney Dis. 2004 Nov;44(5 Suppl 2):54-60.

37. Klip IT, Jankowska EA, Enjuanes C, et al. The additive burden of iron deficiency in the cardiorenal-anaemia axis: scope of a problem and its consequences. Eur J Heart Fail 2014;16:655-62.
38. Collister D, Komenda P, Hiebert B, et al. The Effect of Erythropoietin-Stimulating Agents on Health-Related Quality of Life in Anemia of Chronic Kidney Disease: A Systematic Review and Meta-analysis. Ann Intern Med 2016;164:472-8.
39. Waldvogel-Abramowski S, Waeber G, Gassner C, et al. Physiology of iron metabolism. Transfus Med Hemother 2014;41:213-21.
40. Troosters T. How important is a minimal difference? European Respiratory Journal 2011 37: 755-756
41. Revicki DA, Cella D, Hays RD, Sloan JA, Lenderking WR, Aaronson NK. Responsiveness and minimal important differences for patient reported outcomes. Health Qual Life Outcomes. 2006 Sep 27;4:70.
42. Lopes MB, Tu C, Zee J, et al. A real-world longitudinal study of anemia management in non-dialysis-dependent chronic kidney disease patients: a multinational analysis of CKDopps. Sci Rep 11, 1784 (2021).

Table 1. Patient characteristics by TSAT and ferritin levels

| Characteristics                        | TSAT (%) | Ferritin (ng/mL) |
|----------------------------------------|----------|-----------------|
|                                        | ≤15      | >15-20 | >20-30 | >30-50 | >50   | <50 | 50-99 | 100-299 | ≥300 |
| Number of patients (row %)             |          |        |        |        |       |     |       |        |      |
|                                        | 356 (14%)| 469 (19%)| 1065 (42%)| 580 (23%)| 43 (2%)| 336 (13%)| 572 (23%)| 1156 (46%)| 449 (18%)| 2513 |
| Age (y)                                |          |        |        |        |       |     |       |        |      |
|                                        | 68 ± 12 | 67 ± 13| 68 ± 13| 66 ± 14| 62 ± 15| 66 ± 14| 67 ± 13| 68 ± 13| 67 ± 14| 67 ± 13 |
| Sex (% male)                           |          |        |        |        |       |     |       |        |      |
|                                        | 53%      | 59%    | 63%    | 68%    | 64%   | 49%  | 59%   | 64%    | 68%   | 62%   |
| Race (% Black)                         |          |        |        |        |       |     |       |        |      |
|                                        | 9%       | 7%     | 6%     | 7%     | 15%   | 6%   | 5%    | 6%     | 11%   | 7%    |
| Body mass index (kg/m²)                |          |        |        |        |       |     |       |        |      |
|                                        | 30 ± 7   | 30 ± 7 | 29 ± 6 | 28 ± 6 | 27 ± 5| 29 ± 7| 29 ± 7| 29 ± 6 | 29 ± 6| 29 ± 6 |
| Current smoker (%)                     |          |        |        |        |       |     |       |        |      |
|                                        | 14%      | 11%    | 9%     | 13%    | 17%   | 11%  | 11%   | 12%    | 10%   | 11%   |
| Comorbidities                          |          |        |        |        |       |     |       |        |      |
| Diabetes (%)                           |          |        |        |        |       |     |       |        |      |
|                                        | 56%      | 51%    | 43%    | 33%    | 35%   | 53%  | 44%   | 42%    | 40%   | 44%   |
| Hypertension (%)                       |          |        |        |        |       |     |       |        |      |
|                                        | 94%      | 92%    | 92%    | 87%    | 76%   | 91%  | 89%   | 92%    | 90%   | 91%   |
| Coronary artery disease (%)            |          |        |        |        |       |     |       |        |      |
|                                        | 31%      | 32%    | 25%    | 21%    | 21%   | 29%  | 31%   | 23%    | 26%   | 26%   |
| Heart failure (%)                      |          |        |        |        |       |     |       |        |      |
|                                        | 20%      | 18%    | 13%    | 10%    | 9%    | 15%  | 14%   | 12%    | 17%   | 14%   |
| Cerebrovascular disease (%)            |          |        |        |        |       |     |       |        |      |
|                                        | 13%      | 12%    | 11%    | 10%    | 5%    | 14%  | 11%   | 11%    | 11%   | 11%   |
| Peripheral vascular disease (%)        |          |        |        |        |       |     |       |        |      |
|                                        | 26%      | 24%    | 21%    | 17%    | 13%   | 21%  | 22%   | 21%    | 21%   | 21%   |
| Other cardiovascular disease (%)       |          |        |        |        |       |     |       |        |      |
|                                        | 33%      | 25%    | 26%    | 24%    | 10%   | 27%  | 25%   | 25%    | 29%   | 26%   |
| Characteristics                        | TSAT (%)          | Ferritin (ng/mL) |          |          |          |          |          |          |
|---------------------------------------|-------------------|-----------------|----------|----------|----------|----------|----------|----------|
|                                       | ≤15               | >15-20          | >20-30   | >30-50   | >50      | <50      | 50-99    | 100-299  |
| Gastrointestinal bleeding (%)         | 2%                | 1%              | 1%       | 1%       | 0%       | 2%       | 1%       | 1%       | 2%       | 1%       |
| Lung disease (%)                      | 14%               | 14%             | 9%       | 8%       | 0%       | 10%      | 11%      | 11%      | 11%      | 11%      |
| Cancer (%)                            | 20%               | 19%             | 22%      | 20%      | 24%      | 18%      | 21%      | 21%      | 20%      | 21%      |
| Neurologic disease (%)                | 3%                | 2%              | 3%       | 4%       | 3%       | 2%       | 2%       | 3%       | 4%       | 3%       |
| Psychiatric disorder (%)              | 11%               | 12%             | 9%       | 12%      | 6%       | 10%      | 11%      | 10%      | 10%      | 10%      |
| Ulcers/gangrene of extremity (%)      | 3%                | 3%              | 2%       | 2%       | 0%       | 2%       | 3%       | 3%       | 2%       |          |
| Prescriptions                         |                   |                 |          |          |          |          |          |          |          |
| ESA (%)                               | 12%               | 10%             | 9%       | 12%      | 22%      | 7%       | 7%       | 10%      | 18%      | 10%      |
| IV iron (%)                           | 5%                | 3%              | 2%       | 2%       | 7%       | 2%       | 2%       | 3%       | 5%       | 3%       |
| Oral iron (%)                         | 23%               | 19%             | 12%      | 12%      | 27%      | 14%      | 14%      | 14%      | 20%      | 15%      |
| Any iron (%)                          | 27%               | 21%             | 13%      | 13%      | 31%      | 16%      | 15%      | 16%      | 22%      | 17%      |
| Labs                                  |                   |                 |          |          |          |          |          |          |          |
| TSAT (%)                              | 12 ± 3            | 18 ± 1          | 25 ± 3   | 37 ± 5   | 61 ± 11  | 20 ± 10  | 23 ± 9   | 26 ± 9   | 29 ± 12  | 25 ± 10  |
| Ferritin (ng/mL)                      | 135 ± 175         | 161 ± 186       | 200 ± 195| 239 ± 213| 407 ± 419| 31 ± 11  | 74 ± 15  | 177 ± 55 | 527 ± 282| 196 ± 206|
| Hemoglobin (g/dL)                     | 11.8 ± 1.8        | 12.4 ± 1.6      | 12.8 ± 1.8| 13.1 ± 1.9| 12.3 ± 2.5| 12.6 ± 1.7| 12.8 ± 1.8| 12.8 ± 1.8| 12.2 ± 1.9| 12.6 ± 1.8|
| eGFR (mL/min/1.73m²)                  | 30 ± 12           | 30 ± 11         | 30 ± 12  | 31 ± 12  | 30 ± 13  | 32 ± 11  | 31 ± 12  | 30 ± 12  | 29 ± 12  | 30 ± 12  |
| Albuminuria (%)                       |                   |                 |          |          |          |          |          |          |          |          |
| A1                                    | 29%               | 26%             | 27%      | 32%      | 20%      | 30%      | 27%      | 29%      | 27%      | 28%      |
| A2                                    | 29%               | 32%             | 31%      | 28%      | 40%      | 30%      | 31%      | 29%      | 33%      | 30%      |
| A3                                    | 43%               | 42%             | 42%      | 40%      | 40%      | 41%      | 43%      | 42%      | 40%      | 42%      |
| Serum albumin (g/dL)                  | 3.9 ± 0.5         | 4.0 ± 0.4       | 4.0 ± 0.4| 4.0 ± 0.4| 4.0 ± 0.4| 4.0 ± 0.4| 4.0 ± 0.5| 4.0 ± 0.4| 4.0 ± 0.4| 4.0 ± 0.4|
| White blood cells (10³ cells/mm³)     | 7.6 ± 2.5         | 7.3 ± 2.1       | 6.9 ± 1.9| 6.7 ± 1.8| 7.2 ± 2.9| 7.2 ± 1.9| 7.0 ± 2.1| 7.0 ± 2.0| 7.1 ± 2.3| 7.0 ± 2.1|
| Platelets (10³ cells/mm³)             | 239 ± 78          | 228 ± 67        | 222 ± 62 | 217 ± 60 | 220 ± 69 | 240 ± 66 | 224 ± 64 | 224 ± 66 | 214 ± 62 | 224 ± 65 |
| Systolic blood pressure (mmHg)        | 140 ± 20          | 141 ± 21        | 142 ± 21 | 140 ± 19 | 138 ± 19 | 139 ± 20 | 141 ± 22 | 142 ± 19 | 140 ± 20 | 141 ± 20 |
| Diastolic blood pressure (mmHg)       | 75 ± 12           | 77 ± 12         | 78 ± 12  | 78 ± 11  | 78 ± 10  | 76 ± 12  | 78 ± 12  | 78 ± 12  | 77 ± 11  | 77 ± 12  |
Results reported as % or mean ± SD. 
Patients are from France (N=2015), the United States (N=293), and Brazil (N=205). A1: albuminuria < 30 mg/g creatinine. A2: albuminuria < 300 mg/g creatinine. A3: albuminuria > 300 mg/g creatinine.
Table 2. Patient-reported outcomes by TSAT and ferritin levels

| Characteristics                | TSAT (%) | Ferritin (ng/mL) |
|-------------------------------|----------|-----------------|
|                               | ≤15      | >15-20          |
|                               | >20-30   | >30-50          |
|                               | >50      | <50             |
| Number of patients            |          |                 |
| (row %)                       |          |                 |
| All                           | 356 (14%)| 469 (19%)       |
|                               | 1065 (42%)| 580 (23%)       |
|                               | 43 (2%)  |                 |
|                               | 336 (13%)| 572 (23%)       |
|                               | 1156 (46%)| 449 (18%)       |
| KDQOL-36                      |          |                 |
| MCS                           | 44 ± 11  | 44 ± 11         |
|                               | 45 ± 11  | 46 ± 11         |
|                               | 45 ± 11  | 45 ± 11         |
|                               | 45 ± 11  | 45 ± 11         |
|                               | 45 ± 11  | 45 ± 11         |
| PCS                           | 37 ± 10  | 40 ± 10         |
|                               | 41 ± 10  | 42 ± 10         |
|                               | 41 ± 10  | 40 ± 10         |
|                               | 40 ± 10  | 41 ± 10         |
| General health                | 40 ± 25  | 44 ± 23         |
|                               | 43 ± 24  | 44 ± 24         |
|                               | 46 ± 23  | 47 ± 23         |
|                               | 49 ± 23  | 50 ± 23         |
| Physical function             | 49 ± 35  | 56 ± 33         |
|                               | 62 ± 33  | 62 ± 33         |
|                               | 53 ± 33  | 59 ± 34         |
| Physical role                 | 45 ± 31  | 49 ± 30         |
|                               | 55 ± 31  | 58 ± 30         |
|                               | 57 ± 30  | 61 ± 31         |
| Emotional role                | 55 ± 32  | 57 ± 31         |
|                               | 62 ± 30  | 63 ± 30         |
|                               | 57 ± 30  | 61 ± 31         |
| Pain                          | 55 ± 30  | 60 ± 29         |
|                               | 64 ± 30  | 67 ± 29         |
|                               | 59 ± 29  | 63 ± 28         |
| Emotional well-being          | 62 ± 22  | 64 ± 22         |
|                               | 67 ± 21  | 67 ± 18         |
|                               | 62 ± 22  | 65 ± 21         |
| Energy                        | 36 ± 25  | 42 ± 25         |
|                               | 45 ± 25  | 46 ± 24         |
|                               | 40 ± 25  | 42 ± 25         |
|                               | 43 ± 25  | 43 ± 25         |
| Social function               | 66 ± 29  | 68 ± 27         |
|                               | 70 ± 28  | 70 ± 29         |
|                               | 68 ± 29  | 69 ± 28         |
| Burden of kidney disease      | 69 ± 27  | 72 ± 25         |
|                               | 73 ± 25  | 74 ± 25         |
|                               | 70 ± 26  | 74 ± 24         |
| Symptoms of kidney disease    | 72 ± 17  | 74 ± 16         |
|                               | 76 ± 16  | 78 ± 16         |
|                               | 73 ± 17  | 75 ± 16         |
| Effects of kidney disease     | 76 ± 21  | 79 ± 20         |
|                               | 81 ± 18  | 83 ± 17         |
|                               | 81 ± 18  | 80 ± 19         |
| Other PROs                    |          |                 |
| Physical activity level       |          |                 |
| Low                           | 53%      | 48%             |
|                               | 44%      | 53%             |
|                               | 49%      | 49%             |
|                               | 49%      | 51%             |
| Moderate                      | 27%      | 23%             |
|                               | 27%      | 26%             |
|                               | 27%      | 24%             |
|                               | 24%      | 27%             |
| Intense                       | 19%      | 22%             |
|                               | 29%      | 21%             |
|                               | 24%      | 27%             |
| CES-D 10 score                | 9 ± 6    | 8 ± 5           |
|                               | 8 ± 5    | 7 ± 5           |
|                               | 8 ± 5    | 8 ± 5           |
| Highest status functional     | 41%      | 45%             |
|                               | 49%      | 51%             |
|                               | 45%      | 50%             |
| Results reported as % or mean ± SD. Symptoms of kidney disease is based only on US and French data. Physical activity results based only on French data. MCS: Mental composite summary. PCS: Physical composite summary, PRO: patient-reported outcomes.
Figure Legends

Figure 1. Mean differences in PCS and MCS by TSAT and ferritin levels, with and without adjustment for Hgb

Models were adjusted for country, age, sex, Black race, body mass index, current smoker, estimated glomerular filtration rate, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive heart failure, other cardiovascular disease, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene, and erythropoietin stimulating agents. Additionally, models with TSAT exposure were adjusted for ferritin, and vice versa.

Figure 2. Mean differences in PCS and MCS by combined TSAT/ferritin categories, with and without adjustment for Hgb

Models were adjusted for country, age, sex, Black race, body mass index, current smoker, estimated glomerular filtration rate, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive heart failure, other cardiovascular disease, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene, and erythropoietin stimulating agents.

Figure 3. Mean differences in the probability (%) of highest functional status and intense physical activity by TSAT and ferritin levels, with and without adjustment for Hgb

Models were adjusted for country, age, sex, Black race, body mass index, current smoker, estimated glomerular filtration rate, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive heart failure, other cardiovascular disease, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene, and erythropoietin stimulating agents. Additionally, models with TSAT exposure were adjusted for ferritin, and vice versa.
| HRQOL outcome | Exposure | Mean difference (95% CI) in HRQOL | Mean difference (95% CI) in HRQOL |
|---------------|----------|-----------------------------------|-----------------------------------|
|               |          | Not adjusted for Hgb               | Adjusted for Hgb                  |
| T3AT          | ≤ 15%    | (Ref)                             | (Ref)                             |
|               | > 15-20% | (Ref)                             | (Ref)                             |
|               | > 20-30% | (Ref)                             | (Ref)                             |
|               | > 30-50% | (Ref)                             | (Ref)                             |
| PCS           | ≤ 15%    | (Ref)                             | (Ref)                             |
|               | > 15-20% | (Ref)                             | (Ref)                             |
|               | > 20-30% | (Ref)                             | (Ref)                             |
|               | > 30-50% | (Ref)                             | (Ref)                             |
| MCS           | ≤ 15%    | (Ref)                             | (Ref)                             |
|               | > 15-20% | (Ref)                             | (Ref)                             |
|               | > 20-30% | (Ref)                             | (Ref)                             |
|               | > 30-50% | (Ref)                             | (Ref)                             |
| Ferritin      | < 50 ng/mL | (Ref)                             | (Ref)                             |
|               | 50-99 ng/mL | (Ref)                             | (Ref)                             |
|               | 100-299 ng/mL | (Ref)                             | (Ref)                             |
|               | ≥ 300 ng/mL | (Ref)                             | (Ref)                             |
| PCS           | < 50 ng/mL | (Ref)                             | (Ref)                             |
|               | 50-99 ng/mL | (Ref)                             | (Ref)                             |
|               | 100-299 ng/mL | (Ref)                             | (Ref)                             |
|               | ≥ 300 ng/mL | (Ref)                             | (Ref)                             |

Worse HRQOL
| HRQOL outcome | Exposure | Mean difference (95% CI) in HRQOL | Mean difference (95% CI) in HRQOL |
|---------------|----------|-----------------------------------|-----------------------------------|
|               | TSAT     | Not adjusted for Hgb               | Adjusted for Hgb                  |
| PCS           | ≤ 15%    | (Ref)                             | (Ref)                             |
|               | > 15–20% |                                   |                                   |
|               | > 20–30% |                                   |                                   |
|               | > 30–50% |                                   |                                   |
| MCS           | ≤ 15%    | (Ref)                             | (Ref)                             |
|               | > 15–20% |                                   |                                   |
|               | > 20–30% |                                   |                                   |
|               | > 30–50% |                                   |                                   |
| Ferritin      | PCS      |                                   |                                   |
|               | ≤ 50 ng/mL | (Ref)                           | (Ref)                             |
|               | 50–99 ng/mL |                                 |                                   |
|               | 100–299 ng/mL |                               |                                   |
|               | ≥ 300 ng/mL |                                 |                                   |
|               | MCS      |                                   |                                   |
|               | ≤ 50 ng/mL | (Ref)                           | (Ref)                             |
|               | 50–99 ng/mL |                                 |                                   |
|               | 100–299 ng/mL |                               |                                   |
|               | ≥ 300 ng/mL |                                 |                                   |

Note: The diagram shows the mean difference in HRQOL for different exposures and outcomes, with 95% confidence intervals. The worse HRQOL is indicated by negative values on the x-axis.
| HRQOL outcome | TSAT ≤ 20% | Ferritin | Mean difference (95% CI) in HRQOL | Mean difference (95% CI) in HRQOL |
|---------------|------------|----------|----------------------------------|----------------------------------|
| PCS           |            | < 50 ng/mL | [Diagram]                        | [Diagram]                        |
|               |            | 50–299 ng/mL | [Diagram]                        | [Diagram]                        |
|               |            | ≥ 300 ng/mL  | [Diagram]                        | [Diagram]                        |
|               | > 20–30%   | < 50 ng/mL  | (Ref)                            | [Diagram]                        |
|               |            | 50–299 ng/mL | [Diagram]                        | [Diagram]                        |
|               |            | ≥ 300 ng/mL  | [Diagram]                        | [Diagram]                        |
|               | > 30–50%   | < 50 ng/mL  | [Diagram]                        | [Diagram]                        |
|               |            | 50–299 ng/mL | [Diagram]                        | [Diagram]                        |
|               |            | ≥ 300 ng/mL  | [Diagram]                        | [Diagram]                        |
| MCS           | ≤ 20%      | < 50 ng/mL  | [Diagram]                        | [Diagram]                        |
|               |            | 50–299 ng/mL | [Diagram]                        | [Diagram]                        |
|               |            | ≥ 300 ng/mL  | [Diagram]                        | [Diagram]                        |
|               | > 20–30%   | < 50 ng/mL  | (Ref)                            | [Diagram]                        |
|               |            | 50–299 ng/mL | [Diagram]                        | [Diagram]                        |
|               |            | ≥ 300 ng/mL  | [Diagram]                        | [Diagram]                        |
|               | > 30–50%   | < 50 ng/mL  | [Diagram]                        | [Diagram]                        |
|               |            | 50–299 ng/mL | [Diagram]                        | [Diagram]                        |
|               |            | ≥ 300 ng/mL  | [Diagram]                        | [Diagram]                        |

161x109mm (300 x 300 DPI)
| HRQOL outcome | TSAT  | Ferritin          | Mean difference (95% CI) in HRQOL Not adjusted for Hgb | Mean difference (95% CI) in HRQOL Adjusted for Hgb |
|---------------|-------|-------------------|--------------------------------------------------------|-----------------------------------------------------|
|               |       |                   |                                                        |                                                     |
| PCS ≤ 20%     | < 50 ng/mL | 50–299 ng/mL | ≥ 300 ng/mL | Worse HRQOL | -6 -4 -2 0 2 4 | Worse HRQOL | -6 -4 -2 0 2 4 |
|               | > 20–30% | < 50 ng/mL | 50–299 ng/mL | ≥ 300 ng/mL | Worse HRQOL | -6 -4 -2 0 2 4 | Worse HRQOL | -6 -4 -2 0 2 4 |
|               | > 30–50% | < 50 ng/mL | 50–299 ng/mL | ≥ 300 ng/mL | Worse HRQOL | -6 -4 -2 0 2 4 | Worse HRQOL | -6 -4 -2 0 2 4 |
| MCS ≤ 20%     | < 50 ng/mL | 50–299 ng/mL | ≥ 300 ng/mL | Worse HRQOL | -6 -4 -2 0 2 4 | Worse HRQOL | -6 -4 -2 0 2 4 |
|               | > 20–30% | < 50 ng/mL | 50–299 ng/mL | ≥ 300 ng/mL | Worse HRQOL | -6 -4 -2 0 2 4 | Worse HRQOL | -6 -4 -2 0 2 4 |
|               | > 30–50% | < 50 ng/mL | 50–299 ng/mL | ≥ 300 ng/mL | Worse HRQOL | -6 -4 -2 0 2 4 | Worse HRQOL | -6 -4 -2 0 2 4 |
| HRQOL outcome | Exposure                  | Mean difference (95% CI) in HRQOL |
|---------------|---------------------------|-----------------------------------|
|               |                           | Not adjusted for High             | Adjusted for High                 |
| TSAT          | Highest functional status | ≤ 15%                             | (Ref)                             |
|               |                           | > 15–20%                          | (Ref)                             |
|               |                           | > 20–30%                          | (Ref)                             |
|               |                           | > 30–50%                          | (Ref)                             |
|               | Intense vs. low/moderate  | ≤ 15%                             | (Ref)                             |
|               | physical activity         | > 15–20%                          | (Ref)                             |
|               |                           | > 20–30%                          | (Ref)                             |
|               |                           | > 30–50%                          | (Ref)                             |
| Fontillin     | Highest functional status | < 50 ng/mL                        | (Ref)                             |
|               |                           | 50–99 ng/mL                       | (Ref)                             |
|               |                           | ≥ 100–299 ng/mL                   | (Ref)                             |
|               |                           | ≥ 300 ng/mL                       | (Ref)                             |
|               | Intense vs. low/moderate  | < 50 ng/mL                        | (Ref)                             |
|               | physical activity         | 50–99 ng/mL                       | (Ref)                             |
|               |                           | ≥ 100–299 ng/mL                   | (Ref)                             |
|               |                           | ≥ 300 ng/mL                       | (Ref)                             |
