Nephrol Dial Transplant (2021) 36: 1694–1703
doi: 10.1093/ndt/gfab050
Advance Access publication 24 February 2021

ORIGINAL ARTICLE

Serum biomarkers of iron stores are associated with worse physical health-related quality of life in nondialysis-dependent chronic kidney disease patients with or without anemia

Murilo Guedes[1,2], Daniel Muenz[1], Jarcy Zee[1], Marcelo Barreto Lopes[1], Sandra Waechter[3], Bénédicte Stengel[4], Ziad A. Massy[4,5], Elodie Speyer [4], Carole Ayav[4], Fredric Finkelstein[6], Ricardo Sesso[7], Ronald L. Pisoni[1], Bruce M. Robinson[1] and Roberto Pecoits-Filho[1,2]

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

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

ABSTRACT

Background. Iron deficiency (ID) is a common condition in nondialysis-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 Stages 3–5 patients to test the association between transferrin saturation (TSAT) index and ferritin with HRQoL.

Methods. Patients from Brazil ($n = 205$), France ($n = 2015$) and the USA ($n = 293$) in the Chronic Kidney Disease Outcomes and Practice Patterns Study (CKDopps, 2013–2019) were included. We evaluated the association of TSAT and ferritin (and functional and absolute ID, defined as TSAT $\leq$20% and ferritin $\geq$300 or $<$50 ng/mL) on pre-specified HRQoL measures, including the 36-item Kidney Disease Quality of Life physical component summary (PCS) and mental component summary (MCS) as the primary outcomes. Models were adjusted for confounders including hemoglobin (Hb).

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

Conclusions. Low TSAT levels, as well as both low TSAT with low ferritin and low TSAT with high ferritin, are associated with worse physical HRQoL in NDD-CKD patients, even after accounting for Hb 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

INTRODUCTION

In biological systems, iron exerts essential functions and can potentially result in cell toxicity, particularly in the context of increased intracellular iron content resulting in oxidative injury, which explains the complex regulation of iron metabolism in human physiology [1]. On the other hand, a decrease in iron availability in the body results not only in iron deficiency (ID) 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 [2].

A precise diagnosis of 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 [3]. Although far from ideal, the most commonly used parameters to assess iron status in clinical practice are transferrin saturation (TSAT) and ferritin. Based on these biochemical parameters, there are two main ID subtypes: absolute ID, loosely defined as low TSAT

KEY LEARNING POINTS

What is already known about this subject?

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

What this study adds?

- In nondialysis-dependent chronic kidney disease (NDD-CKD) patients, clinical trials in ID have been limited to erythropoetic-focused goals, lacking PROs. We sought to explore the associations between iron stores and PROs among NDD-CKD patients with or without anemia.
- Our description of the associations between ID and physical aspects of health-related quality of life 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, resulting in patient-reported adverse outcomes.

What impact this may have on practice or policy?

- Our results support the need for randomized controlled trials (RCTs) 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 RCTs may further test this hypothesis and help foster a more patient-centered approach to ID management in CKD care.

and low ferritin levels, and functional ID, defined as 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) [4]. 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 [5].

ID is a common finding in nondialysis-dependent CKD (NDD-CKD), occurring in up to 50% of patients with anemia [3, 6, 7]. 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 underevaluated and undertreated for ID [8, 9].

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, independent from anemia, has been associated with worse functional and patient-reported outcomes (PROs) in observational studies [10, 11]. Randomized controlled trials (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 health-related quality of life (HRQoL), and particularly independent from anemic states, has not been previously investigated. We therefore designed an analysis using data from chronic kidney disease outcomes and practice patterns study (CKDopps) [18], an ongoing international prospective cohort study of adult NDD-CKD patients, to address the following hypotheses: 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 the association between serum biomarkers of iron stores and HRQoL is not mediated or modified by hemoglobin (Hb) levels.

MATERIALS AND METHODS

Patient sample

CKDopps is an ongoing prospective cohort study of Stages 3–5 NDD-CKD patients treated in nephrologist-led CKD clinics in Brazil, France, Germany, Japan and the USA. CKDopps sites were randomly selected from CKD clinics after stratification by region. CKDopps study design, details and objectives have previously been published [18]. 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 compared with 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 imputations 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. The median days from exposure to HRQoL data was 24 (interquartile range 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, 50–99, 100–299 (reference group) and ≥300 ng/mL. We also considered joint categories of TSAT and ferritin, defining ferritin as <50, 50–299 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 a sufficient sample size within each category.

Outcomes

Primary outcome. The 36-item kidney disease quality of life (KDQOL-36) questionnaire combines both general HRQoL measures and kidney-specific domains [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 [21]. For this analysis we created a binary outcome combining the low and moderate categories [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 [23]. We followed the standard procedure of scoring each item from 0 to 3 and summing to create a single continuous score from 0 to 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 ADL (IADL) Lawton–Brody scale; both were previously validated in the general population [24, 25]. In brief, these instruments assess the respondent's independence for performing a set of activities (e.g. eating, getting dressed and doing laundry) and are strongly correlated with poor prognosis and more healthcare system use both in the general population and in end-stage renal disease 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. We calculated scores for the eight MCS and PCS 12-item Short Form Health Survey (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 the 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 to 3, indicating the presence of coronary artery disease, cerebrovascular disease and/or peripheral vascular disease), congestive HF, other cardiovascular diseases, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene and erythropoietin-stimulating agent (ESA) prescription. Two sets of models, one with and one without adjustment for Hb level, were constructed to assess whether Hb could be a potential mediator of the effect of ID on HRQoL. Hb 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 versus 4 and 5), iron treatment (any intravenous or oral iron versus none in the 6 months before the exposure) and anemia status (defined as Hb <13.5 g/dL in men and <12 g/dL in women).

Multiple imputations, implemented by IVEware [27], were 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 [28].

All analyses used SAS software version 9.4 (SAS Institute, Cary, NC, USA). Confidence intervals (CIs) are reported with a 95% confidence level.

RESULTS

A total of 2513 NDD-CKD patients from France [$n = 2015$ (80%)], Brazil [$n = 205$ (8%)] and the USA [$n = 293$ (12%)] were eligible for inclusion in this analysis (Supplementary data, Figure S1). 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 and had lower BMI, higher serum ferritin levels and a 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

Table 1. Patient characteristics by TSAT and ferritin levels

Characteristics	TSAT (%)					Ferritin (ng/mL)				All
	≤15	>15–20	>20–30	>30–50	>50	<50	50–99	100–299	≥300	
Patients, *n* (%)	356 (14)	469 (19)	1065 (42)	580 (23)	43 (2%)	336 (13)	572 (23)	1156 (46)	449 (18)	2513
Age (years), mean ± SD	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
BMI (kg/m^2), mean ± SD	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
HF	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
Gastrointestinal bleeding	2	1	1	1	0	2	1	1	2	1
Lung disease	14	14	9	8	8	10	11	11	10	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	2	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, mean ± SD										
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
Hb (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/1.7 m^2)	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), mean ± SD	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 (10^3 cells/mm^3), mean ± SD	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 (10^3 cells/mm^3), mean ± SD	239 ± 78	228 ± 67	222 ± 62	217 ± 60	220 ± 69	240 ± 66	224 ± 64	224 ± 66	214 ± 62	224 ± 65
Systolic blood pressure (mmHg), mean ± SD	140 ± 20	141 ± 21	142 ± 21	140 ± 19	138 ± 19	139 ± 20	141 ± 22	142 ± 19	140 ± 20	141 ± 20
Diastolic blood pressure (mmHg), mean ± SD	75 ± 12	77 ± 12	78 ± 12	78 ± 11	78 ± 10	76 ± 12	78 ± 12	78 ± 12	77 ± 11	77 ± 12

Patients are from France ($n = 2015$), the USA ($n = 293$) and Brazil ($n = 205$). A1: albuminuria <30 mg/g creatinine; A2: albuminuria <300 mg/g creatinine; A3: albuminuria >300 mg/g creatinine.

TSAT levels. The mean eGFR was similar across TSAT groups. Regarding ferritin, there were 13, 23, 46 and 18% of persons distributed in ferritin categories of <50, 50–99, 100–299 and ≥300 ng/mL. Differences in patient characteristics across ferritin categories were less pronounced compared with 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 Supplementary data, Table S1.

The 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 PROs are shown in Table 2.

Patients with TSAT ≤15% had a −1.8 (95% CI −3.1 to −0.5) adjusted mean difference in PCS and a −0.7 (95% CI −2.2–0.7) mean difference in MCS compared with patients with TSAT >20–30% (Figure 1). Patients with ferritin ≥300 ng/mL had a −1.3 (95% CI −2.4 to −0.3) mean difference for PCS and 0.2 (95% CI −1.0–1.5) for MCS compared with the reference ferritin group of 100–299 ng/mL, while those with ferritin <50 ng/mL had a −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 Hb only slightly attenuated the observed effect sizes. Furthermore, similar results were seen in subgroup analyses stratified according to CKD stage, including for

Table 2. PROs by TSAT and ferritin levels

Characteristics	TSAT (%)					Ferritin (ng/mL)				All
	≤15	>15–20	>20–30	>30–50	>50	<50	50–99	100–299	≥300	
Patients, *n* (%)	356 (14)	469 (19)	1065 (42)	580 (23)	43 (2)	336 (13)	572 (23)	1156 (46)	449 (18)	2513
KDQOL-36, mean ± SD										
MCS	44 ± 11	44 ± 11	45 ± 11	46 ± 11	45 ± 11	44 ± 11	45 ± 11	45 ± 11	45 ± 11	45 ± 11
PCS	37 ± 10	40 ± 10	41 ± 10	42 ± 10	41 ± 10	39 ± 10	41 ± 11	41 ± 10	40 ± 11	41 ± 10
General health	40 ± 25	44 ± 23	48 ± 24	49 ± 24	42 ± 24	43 ± 24	46 ± 24	48 ± 23	46 ± 24	46 ± 24
Physical function	49 ± 35	56 ± 33	61 ± 34	62 ± 33	62 ± 33	53 ± 33	59 ± 34	61 ± 34	56 ± 35	59 ± 34
Physical role	45 ± 31	49 ± 30	55 ± 31	58 ± 30	55 ± 31	49 ± 30	53 ± 30	55 ± 31	51 ± 32	53 ± 31
Emotional role	55 ± 32	57 ± 31	62 ± 30	63 ± 30	58 ± 30	57 ± 30	61 ± 30	61 ± 31	60 ± 32	60 ± 31
Pain	55 ± 30	60 ± 29	64 ± 30	67 ± 29	63 ± 28	59 ± 29	63 ± 30	64 ± 30	62 ± 30	63 ± 30
Emotional well-being	62 ± 22	64 ± 22	65 ± 21	67 ± 21	67 ± 18	62 ± 22	65 ± 21	65 ± 21	67 ± 21	65 ± 21
Energy	36 ± 25	42 ± 25	44 ± 26	45 ± 25	46 ± 24	40 ± 25	42 ± 25	44 ± 25	43 ± 26	43 ± 25
Social function	66 ± 29	68 ± 27	70 ± 28	70 ± 29	66 ± 29	68 ± 29	69 ± 28	69 ± 28	68 ± 28	69 ± 28
Burden of kidney disease, mean ± SD	69 ± 27	72 ± 25	73 ± 25	74 ± 25	71 ± 28	70 ± 26	74 ± 24	73 ± 25	71 ± 27	73 ± 25
Symptoms of kidney disease, mean ± SD	72 ± 17	74 ± 16	76 ± 16	78 ± 16	75 ± 16	73 ± 17	75 ± 17	77 ± 16	75 ± 17	76 ± 17
Effects of kidney disease, mean ± SD	76 ± 21	79 ± 20	81 ± 18	83 ± 17	79 ± 18	81 ± 18	80 ± 19	81 ± 18	80 ± 19	81 ± 19
Other PROs										
Physical activity level,%										
Low	53	54	48	44	53	49	49	49	51	49
Moderate	27	23	23	27	26	27	24	24	23	24
Intense	19	22	29	29	21	24	27	27	26	27
CES-D 10 score, mean ± SD	9 ± 6	8 ± 5	8 ± 5	7 ± 5	8 ± 5	8 ± 5	8 ± 5	8 ± 5	8 ± 5	8 ± 5
Highest functional status,%	41	45	49	51	64	45	47	50	44	48

Symptoms of kidney disease are based on USA and French data. Physical activity results based on only French data.

patients with Stage 3 versus Stages 4 and 5 CKD, for those prescribed versus not prescribed iron treatment and for those with Hb ≥11.5 versus <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 a −2.3 lower PCS (95% CI −3.9 to −0.8) compared with 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 to −1.6)]. Consistently, adjustment for Hb 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 with 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 (Supplementary data, Figure S2). Regarding ferritin, only small differences were seen in the mean values for these domains (Supplementary data, Figure S2). Investigation of the subdomains of PCS and MCS revealed modest associations for physical-related HRQoL subdomains in relationship to TSAT and ferritin levels (Supplementary data, Figure S3). For TSAT, the subdomain scores of general health, pain, physical function and energy were consistently lower for persons with TSAT <20% (Supplementary data, Figure S3). For ferritin, overall, both higher and lower levels were associated with worse subdomain scores, particularly for physical function, physical role and pain (Supplementary data, Figure S4). Consistent with findings for mental domains, there was little difference in mean CES-D levels across TSAT and ferritin categories (Supplementary data, Figure S5).

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 with patients with TSAT >20–30% and ferritin 50–299 ng/mL. Effect sizes were not materially changed by adjustment for Hb (Supplementary data, Table S2). For self-reported physical activity, patients with TSAT <20% had a lower probability of routinely performing intense physical activity compared with 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, low TSAT and combinations of low TSAT with high and low ferritin were associated with worse PROs, particularly for physical domains of HRQoL. Importantly, these associations were not materially affected by the adjustment for Hb levels and were consistent among subgroups of anemic versus nonanemic individuals, suggesting that the observed effect (whether biologically causal or not) is direct rather than mediated through Hb. 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 <20% and ferritin >300 ng/mL would be representative of functional ID (iron restriction), while TSAT <20% and ferritin <50 ng/mL represented the absolute ID subtype (iron depletion); we considered the fact that the exact cutoffs for such definitions have been highly debated and have demonstrated great variability

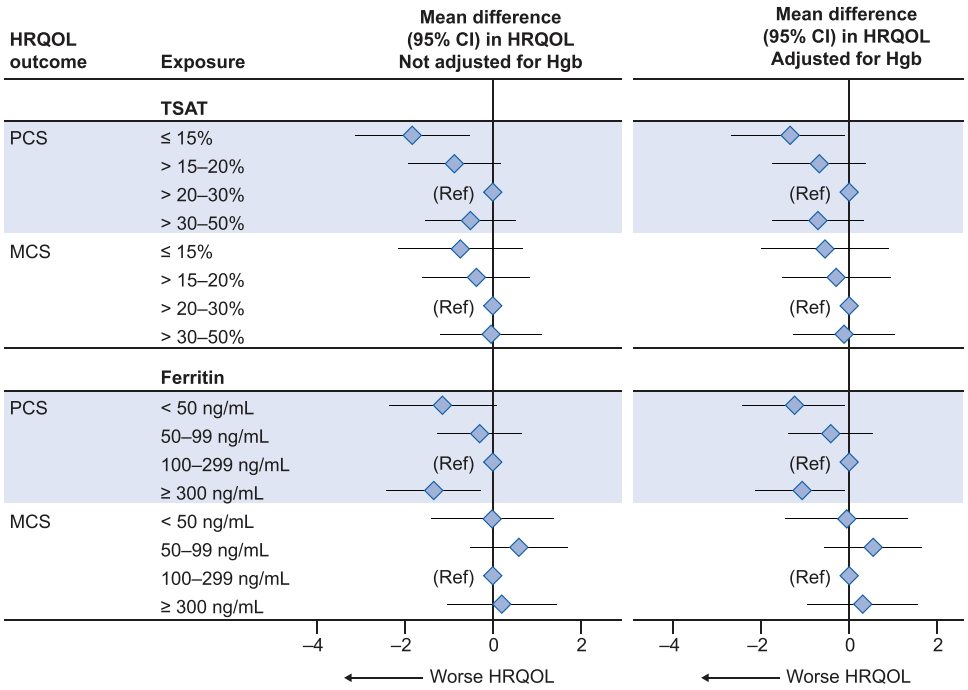

FIGURE 1: Mean differences in PCS and MCS by TSAT and ferritin levels with and without adjustment for Hb. Models were adjusted for country, age, sex, Black race, BMI, current smoker, eGFR, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive HF, other cardiovascular diseases, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene and ESAs. Additionally, models with TSAT exposure were adjusted for ferritin, and vice versa.

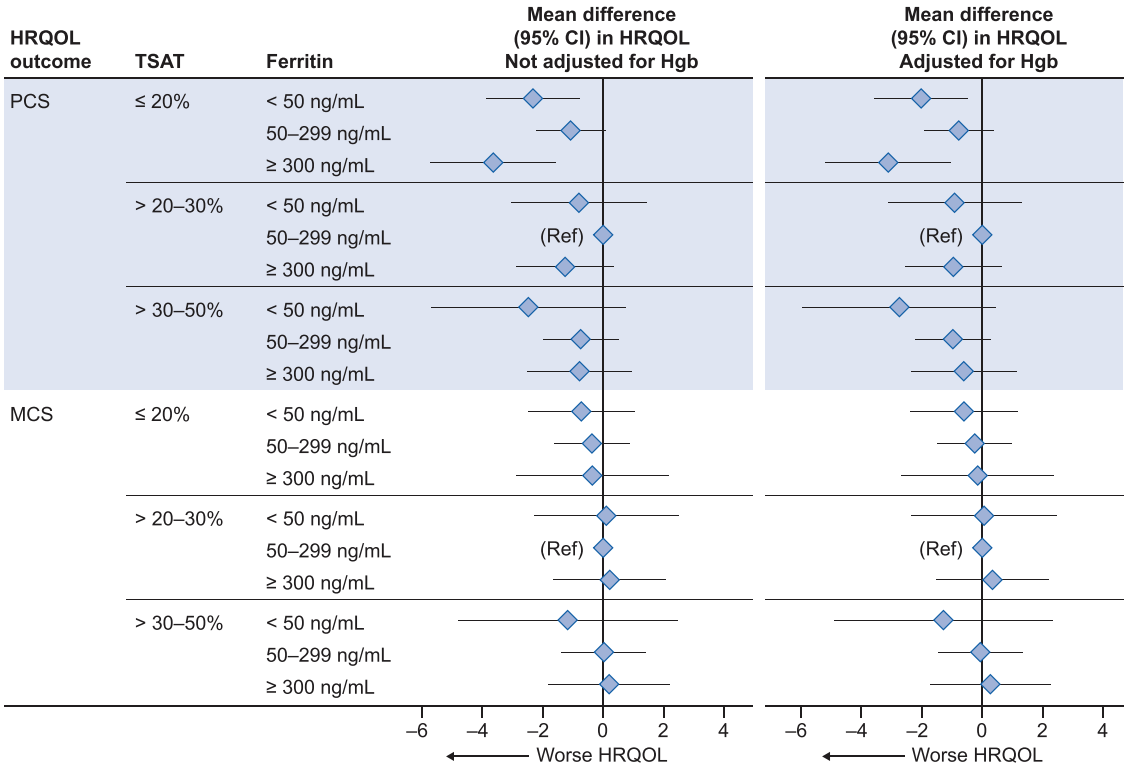

FIGURE 2: Mean differences in PCS and MCS by combined TSAT/ferritin categories, with and without adjustment for Hb. Models were adjusted for country, age, sex, Black race, BMI, current smoker, eGFR, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive HF, other cardiovascular diseases, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene and ESAs.

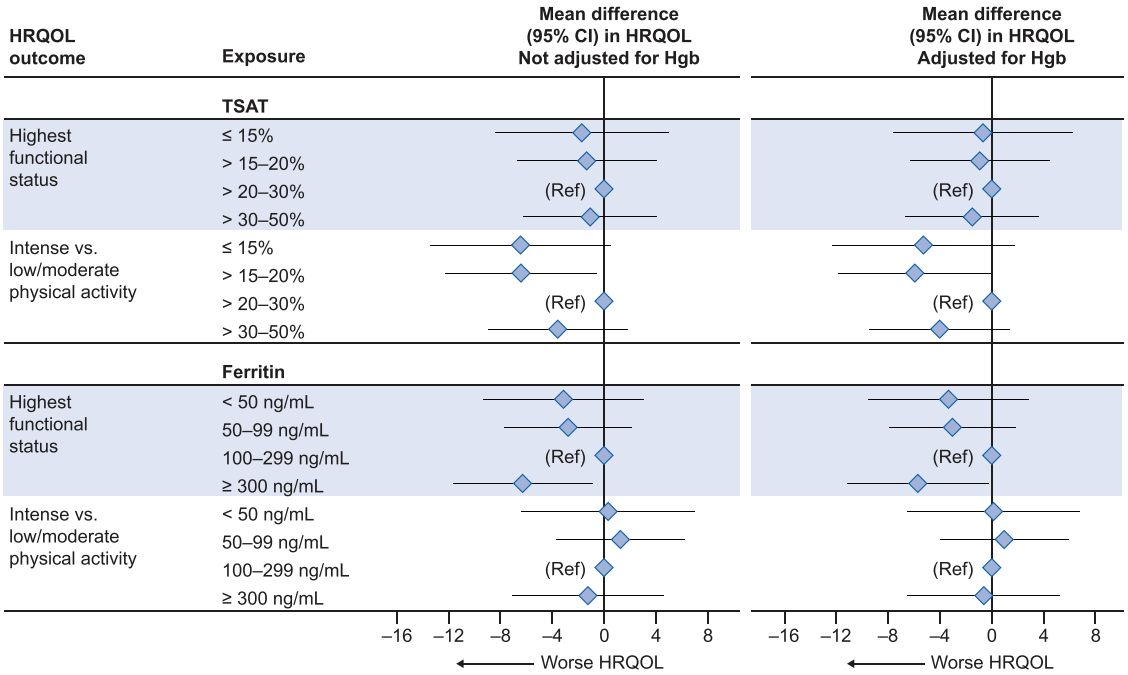

FIGURE 3: Mean differences in the probability (%) of highest functional status and intense physical activity by TSAT and ferritin levels with and without adjustment for Hb. Models were adjusted for country, age, sex, Black race, BMI, current smoker, eGFR, albuminuria, serum albumin, white blood cell count, diabetes, hypertension, atherosclerotic cardiovascular disease score, congestive HF, other cardiovascular diseases, gastrointestinal bleeding, lung disease, cancer, ulcers/gangrene and ESAs. Additionally, models with TSAT exposure were adjusted for ferritin, and vice versa.

across medical specialties [29]. To guide our choice of cutoffs 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. In particular, 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 >300 ng/mL, which is the maximum range for inclusion in these trials, would most likely reflect iron restriction rather than depletion [13, 30]. 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 [9]. This is also consistent with the observation that, in the present analysis, functional ID was higher among those with more advanced CKD, which confirms findings in previous cohort studies [14].

In an analysis of a previous cohort study [14, 15], ID was associated with higher mortality risk among NDD-CKD patients. In particular, patients with functional ID tended to have a slightly higher risk of mortality and cardiovascular events compared with those with absolute ID [14, 15]. 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 ID as a result of a mismatch between iron supply and demand may impact cell function, in a similar condition as in absolute ID [31]. At a cellular level in nonerythropoietic tissues, the impact of an unmet need for 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 PROs and cardiovascular events independent from anemia status, as demonstrated by clinical trials [13, 29, 32, 33]. The potential mechanisms by which reestablishing iron stores could improve outcomes in such patients, independent of its erythropoietic effects, are highlighted by the concept of tissue ID, given that iron is essential for the synthesis of many enzymes for cell energy metabolism, including mitochondrial electron transport chain components [2, 34]. 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 scores, with small effect estimates sustained even after adjustment for Hb. 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 important differences (CIDs), according to studies in patients with CKD anemia and

consistent with previous Dialysis Outcomes and Practice Patterns Study (DOPPS) analyses [35, 36]. 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 ID on muscle and physical function, as has been extensively studied in HF patients [11, 37]. Compared with 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 [5], characterizing functional ID. In fact, we found that the subgroup of patients with high ferritin and low TSAT had generally worse physical PROs compared with absolute ID persons. Notably, higher ferritin levels were strongly associated with a higher risk of having impairment of daily living activities. Our analyses showed that this effect was driven primarily by those patients who also had a TSAT <20%, with a 15% higher probability of worse functionality in this subgroup compared with those with TSAT of 20–30% and ferritin 50–299 ng/mL. Consistently these effect estimates remained after adjustment for Hb, 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 [26]. 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 subdomains 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 [38]. These results further reinforce the hypothesis that ID has a primary impact on physical function, probably through tissue ID mainly affecting energy metabolism in muscle tissues, which could explain the major benefits of iron treatment in physical function in patients with comorbidities such as HF [13, 39].

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, Hb and PROs. We assumed Hb would be a potential mediator of the associations between iron parameters and PROs. Therefore, in our study design, we did not include Hb data preceding the measurement of TSAT/ferritin. Also, we defined at least two sets of fully adjusted models for confounders, one including Hb 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 the 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. Our results for these subdomains are purely exploratory and 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 CID, it is important to consider that methods for determining CIDs can vary according to the disease state, potential intervention and patient population [40, 41]. Potential improvements in PROs, even modest in magnitude, achieved by low-cost or highly available interventions could translate into important population 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 cutoffs for ID [3], as these are generally arbitrary and vary considerably both within nephrology and across different specialties [29]. Therefore the sample prevalence of patients with both low TSAT with low ferritin and low TSAT with 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 [9] and the intense variability in treatment patterns observed in a previous analysis of our cohort [42] 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 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 [9]. It will be important to design intervention studies to analyze the hypothesis raised by our observation (and supported by trials in HF [13, 33]) 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, is associated with worse physical HRQoL in NDD-CKD patients, even after adjustment or stratification by Hb level. RCTs 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 DATA

Supplementary data are available at ndt online.

ACKNOWLEDGEMENTS

CKDopps Steering Committee and Country Investigators: Antonio Lopes and Roberto Pecoits-Filho (Brazil); Christian Combe, Christian Jacquelinet, Ziad Massy and Benedicte Stengel (France); Johannes Duttlinger, Danilo Fliser, Gerhard Lonnemann and Helmut Reichel (Germany); Takashi Wada and Kunihiro Yamagata (Japan); Ron Pisoni and Bruce Robinson (USA). Additional CKDopps Research Group: Viviane Calice da Silva and Ricardo Sesso (Brazil); Elodie Speyer (France); Koichi Asahi, Junichi Hoshino and Ichiei Narita (Japan); Rachel Perlman, Friedrich Port, Nidhi Sukul, Michelle Wong, Eric Young and Jarcy Zee (USA). In France, CKDopps is part of the Chronic Kidney Disease–Renal Epidemiology and Information Network (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 since 2012, Baxter and Merck Sharp & Dohme-Chibret from 2012 to 2017, Lilly France since 2013, Otsuka Pharmaceutical since 2015, Vifor Fresenius since 2017 and Sanofi-Genzyme from 2012 to 2015. In the USA and Brazil, support for the CKDopps Coordinating Center has been provided by Keryx. Jennifer McCready-Maynes, an employee of Arbor Research Collaborative for Health, provided editorial assistance.

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 Pharma.

AUTHORS' CONTRIBUTIONS

R.L.P., M.G., S.W., D.M., R.P.F., B.M.R., Z.M., C.A., J.Z., R.S., E.S. and B.S. conceived and/or designed the work that led to the submission, acquired data and/or played an important role in interpreting the results. R.L.P., M.G., S.W., D.M., R.P.F., B.M.R., Z.M., J.Z., M.B.L., R.S., E.S. and F.F. drafted or revised the manuscript. R.L.P., M.G., S.W., D.M., R.P.F., B.M.R., C.A., J.Z., M.B.L., R.S., E.S., B.S. and F.F. approved the final version. 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 article have not been published previously in whole or part, except in abstract form.

CONFLICT OF INTEREST STATEMENT

M.G., D.M., J.Z., M.B.L., R.L.P., B.M.R. and R.P.-F. 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, B.M.R. has received consultancy fees or travel reimbursement in the last 3 years from AstraZeneca, GlaxoSmithKline and Kyowa Kirin, all paid directly to his institution of employment. R.P.-F. reports grants from Fresenius Medical Care, nonfinancial support from AstraZeneca, Novo Nordisk and Akebia and personal fees from Retrophin, outside the submitted work. Z.M. reports grants and other fees from Amgen, Baxter and Sanofi-Genzyme; grants from the French government, MSD, GlaxoSmithKline, Eli Lilly, FMC and Outsuka and other fees from Daichi and Astellas, outside the submitted work. E.S. reports grants from Vifor Fresenius, Amgen, Otsuka, Fresenius, GlaxoSmithKline and AstraZeneca, during the conduct of the study. B.S. reports grants from Vifor Fresenius, Amgen, Otsuka, Fresenius, GlaxoSmithKline and AstraZeneca, during the conduct of the study. S.W. is an employee of Vifor Fresenius Medical Care. F.F., R.S. and C.A. have nothing to disclose.

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 the data that were used for the current study and thus 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 *et al.* 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. *J Nutr* 2001; 131(2 Suppl): 568S–580S
3. Kidney Disease: Improving Global Outcomes Anemia Work Group. KDIGO clinical practice guideline for anemia in chronic kidney disease. *Kidney Int Suppl* 2012; 2: 279–335
4. De Franceschi L, Iolascon A, Taher A *et al.* 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: 252–261
6. Batchelor EK, Kapitsinou P, Pergola PE *et al.* Iron deficiency in chronic kidney disease: updates on pathophysiology, diagnosis, and treatment. *J Am Soc Nephrol* 2020; 31: 456–468
7. Fishbane S, Spinowitz B. Update on anemia in ESRD and earlier stages of CKD: core curriculum 2018. *Am J Kidney Dis* 2018; 71: 423–435
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: 2237–2244

9. Wong MMY, Tu C, Li Y *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, variably treated. *Clin Kidney J* 2019; 13: 613–624
10. Martens P, Nijst P, Verbrugge FH *et al.* Impact of iron deficiency on exercise capacity and outcome in heart failure with reduced, mid-range and preserved ejection fraction. *Acta Cardiol* 2018; 73: 115–123
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: 2436–2448
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: 125–133
14. Awan AA, Walther CP, Richardson PA *et al.* Prevalence, correlates and outcomes of absolute and functional iron deficiency anemia in nondialysis-dependent chronic kidney disease. *Nephrol Dial Transplant* 2019; 36: 129–136
15. Cho ME, Hansen JL, Peters CB *et al.* An increased mortality risk is associated with abnormal iron status in diabetic and non-diabetic veterans with predialysis chronic kidney disease. *Kid Int* 2019; 96: 750–760
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: 2075–2084.
17. Shepshelovich D, Rozen-Zvi B, Avni T *et al.* Intravenous versus oral iron supplementation for the treatment of anemia in CKD: an updated systematic review and meta-analysis. *Am J Kidney Dis* 2016; 68: 677–690
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: 402–413
19. Hays RD, Kallich JD, Mapes DL *et al.* Development of the Kidney Disease Quality of Life (KDQOL) instrument. *Qual Life Res* 1994; 3: 329–338
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: 220–233
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. World Health Organization. *Global Physical Activity Questionnaire (GPAQ) Analysis Guide*. Geneva: World Health Organization, 2020
23. Andresen EM, Malmgren JA, Carter WB *et al.* 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 *et al.* 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: 179–186
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: 283–292
27. Raghunathan TE, Solenberger PW, Van Hoewyk J. *IVEware: Imputation and variance estimation software.* Ann Arbor, MI: 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: 1068–1078
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; 396: 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; 19: 522–530
32. Bekfani T, Pellicori 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: 203–211
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: 1958–1966
34. Paul BT, Manz DH, Torti FM *et al.* Mitochondria and Iron: current questions. *Expert Rev Hematol* 2017; 10: 65–79
35. Finkelstein FO, van Nooten F, Wiklund I *et al.* 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 Qual Life Outcomes* 2018; 16: 111
36. Mapes DL, Bragg-Gresham JL, Bommer J *et al.* Health-related quality of life in the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Am J Kidney Dis* 2004; 44: 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: 655–662
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: 472–478
39. Waldvogel-Abramowski S, Waeber G, Gassner C *et al.* Physiology of iron metabolism. *Transfus Med Hemother* 2014; 41: 213–221
40. Troosters T. How important is a minimal difference? *Eur Respirat J* 2011; 37: 755–756
41. Revicki DA, Cella D, Hays RD *et al.* Responsiveness and minimal important differences for patient reported outcomes. *Health Qual Life Outcomes* 2006; 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* 2021; 11: 1784.

Received: 6.10.2020; Editorial decision: 11.2.2021