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Original Article

Iron deficiency, anemia, and patient-reported outcomes in kidney transplant recipients

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ABSTRACT

Kidney transplant recipients (KTRs) experience more fatigue, anxiety, and depressive symptoms and lower concentration and health-related quality of life (HRQoL) compared with the general population. Anemia is a potential cause that is well-recognized and treated. Iron deficiency, however, is often unrecognized, despite its potential detrimental effects related to and unrelated to anemia. We investigated the interplay of anemia, iron deficiency, and patient-reported outcomes in 814 outpatient KTRs (62% male, age 56 ± 13 years) enrolled in the TransplantLines Biobank and Cohort Study (Groningen, The Netherlands). In total, 28% had iron deficiency (ie, transferrin saturation < 20% and ferritin < 100 μg/L), and 29% had anemia (World Health Organization criteria). In linear regression analyses, iron deficiency, but not anemia, was associated with more fatigue, worse concentration, lower

Abbreviations: ACE, angiotensin-converting enzyme; CI, confidence interval; CIS20R, Checklist of Individual Strength 20 Revised; CNI, calcineurin inhibitors; eGFR, estimated glomerular filtration rate; EQ5D-3L, EuroQol 5-Dimensions 3-Level; ESA, erythropoietin-stimulating agent; HRQoL, health-related quality of life; KTRs, kidney transplant recipients; mTOR, mammalian target of rapamycin; OR, odds ratio; PHQ-9, Patient Health Questionnaire-9; PPI, proton pump inhibitor; PRO, patient-reported outcome; SF-36, short form 36; STAI-6, 6-item State-Trait Anxiety Inventory; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; TSAT, transferrin saturation; UMCG, University Medical Center Groningen; VAS, visual analog scale; WHO(-5), World Health Organization (-Five).

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depressive symptoms
wellbeing

wellbeing, more anxiety, more depressive symptoms, and lower HRQoL, independent of age, sex, estimated glomerular filtration rate, anemia, and other potential confounders. In the fully adjusted logistic regression models, iron deficiency was associated with an estimated 53% higher risk of severe fatigue, a 100% higher risk of major depressive symptoms, and a 51% higher chance of being at risk for sick leave/work disability. Clinical trials are needed to investigate the effect of iron deficiency correction on patient-reported outcomes and HRQoL in KTRs.

1. Introduction

In recent decades, a growing number of patients have been living with a kidney transplant for increasing periods of time, owing to improvements in graft and patient survival after transplantation.[1] Consequently, life after transplantation—beyond graft and patient survival—has become more important, and the topic receives increasing scientific attention. Unfortunately, KTRs have more psychosocial symptoms, including fatigue, anxiety, and depressive symptoms, compared with the general population.[2] As a result, health-related quality of life (HRQoL) in kidney transplant recipients (KTRs) remains impaired compared with the general population.[2,3] Therefore, there is a need for interventions to improve these patient-reported outcomes (PROs) after kidney transplantation.

An important cause of these impaired PROs and HRQoL in KTRs is anemia.[4,5] Anemia in KTRs is highly prevalent and is associated with poor clinical outcomes in this patient population.[6,7] A main cause of anemia is iron deficiency. Iron deficiency in KTRs is multifactorial, determined by combinations of systemic low-grade inflammation that impairs iron absorption and drives iron toward the intracellular compartment, increased erythropoiesis after transplantation, and use of medications, eg, anticoagulants leading to blood loss and proton pump inhibitors decreasing iron absorption.[8] In recent years, the consequences of iron deficiency beyond anemia have been increasingly acknowledged, as iron fulfills myriad functions besides fueling erythropoiesis. For example, iron is crucial for muscle, brain, and cardiovascular health.[9-11] Hence, iron deficiency may be a promising target, even in the absence of anemia, to improve PROs and HRQoL among KTRs.[5,9] However, Wong et al[12] showed that iron deficiency is currently often disregarded—or even unrecognized—in current nephrology practice, even though it can be safely and effectively treated in KTRs.[13-15] Due to the lack of epidemiological support, iron status assessment in the absence of anemia is currently not explicitly endorsed in treatment guidelines.[5,16] More research on this topic is warranted, as highlighted by a recent Kidney Disease: Improving Global Outcomes (KDIGO) statement, which defined studies addressing the interplay between anemia, iron status, and HRQoL as a research priority.[5]

We hypothesized that iron deficiency is associated with PROs, including individual strength, fatigue, wellbeing, anxiety, depression, and HRQoL, independent of coexisting anemia. In the current study, we therefore investigated the associations of iron deficiency with PROs, including HRQoL, in KTRs, taking into account the potential confounding and mediating roles of anemia.

2. Materials and Methods

This study was written in accordance with the guidelines for Strengthening the Reporting of Observational studies in Epidemiology.[17] The Strengthening the Reporting of Observational studies in Epidemiology checklist is provided in Supplementary Table 1.

2.1. Study population

For this study, we used cross-sectional data from the TransplantLines Biobank and Cohort Study (ClinicalTrials.gov identifier: NCT03272841).[18] This ongoing, single-center, prospective cohort study aims to provide a better understanding of disease-related and aging-related outcomes and health problems, both physical and psychological, in solid organ transplant recipients and living organ donors. From June 2015 on, all potential solid organ transplantation patients and living donors (age $\geq$18 years) of the University Medical Center Groningen (The Netherlands) were invited to participate. The study complies with the University Medical Center Groningen Biobank Regulations, was approved by the local institutional review board (METc 2014/017), and conforms to the declarations of Helsinki and Istanbul. All participants were at least 18 years old at the time of enrollment and provided written informed consent.

For the current analyses, approval was obtained from the Transplantlines Scientific Committee, thereby adhering to the local ethical regulations. We included outpatient KTRs ($\geq$1 year posttransplant) with available iron status parameters (assessed from April 2016 onward) and a complete HRQoL assessment. Data extraction occurred in June 2021. All patients were included only when they had no known ongoing infections, graft rejections, or hospitalizations.

2.2. Outcome definitions

Psychosocial factors that were included were individual strength attributes, wellbeing, anxiety, and symptoms of depression. Individual strength was assessed using the Checklist of Individual Strength 20 Revised (CIS20R), resulting in a fatigue severity, concentration, motivation, physical activity, and total score.[19,20] Fatigue severity was categorized as no-mild (score $<$ 20), moderate (score 20-34), and severe (score $\geq$ 35). A score $>$ 76 on the total Checklist of Individual Strength 20 Revised-score is regarded as being "at risk" for sick leave or

work disability.[21] Wellbeing was assessed using the World Health Organization-Five; a score ≤ 50 is indicative of poor wellbeing.[22] Symptoms of anxiety were investigated using the 6-item State-Trait Anxiety Inventory, applying a cut-off score of ≥ 40 to define the presence of anxiety.[23] Finally, depression was assessed using the Patient Health Questionnaire-9; a Patient Health Questionnaire-9 score ≥ 10 is indicative of major depressive symptoms (sensitivity and specificity of 88% for major depression).[24] HRQoL was assessed using validated Dutch translations of the Short Form 36 (SF-36) and EuroQol Five-Dimensions 3-level (EQ5D-3L) questionnaires.[25-28] For the SF-36, the physical component score was calculated using the mean scores on general health, physical health, role limitations due to impairment of physical health, and pain. The mental component score was calculated using the mean scores on emotional wellbeing, role limitations due to emotional problems, social functioning, and vitality. For the EQ5D-3L, the Dutch value set was used to calculate the utility (EQ5D-3L) score.[29] Questionnaires were sent a maximum of 4 weeks prior to the study visit.

2.3. Exposures and covariables

Exposures and covariables were assessed at the time of the study visit, which was considered baseline. In primary analyses, iron deficiency was defined as proposed by KDIGO, the European Renal Best Practice Group, and the National Institute for Health and Care Excellence NG8 guidelines (ie, both transferrin saturation [TSAT] levels $< 20\%$ and ferritin levels < 100 μg/L).[5,30,31] In sensitivity analyses, we repeated the analyses regarding associations with HRQoL using an alternative iron deficiency definition of both TSAT levels $< 20\%$ and ferritin levels < 300 μg/L, which was previously used in KTRs.[32,33] Anemia was defined as a hemoglobin level < 13 g/dL (for males) or < 12 g/dL (for females) according to the World Health Organization and KDIGO definitions.[34,35] Assessment of iron status and hemoglobin level was protocolized in patients enrolled in this prospective cohort study, limiting the risk of confounding by indication.

Clinical and transplant-related parameters were retrieved from patient medical files. Medication use was retrieved from patient medical files and verified with the patients during a study visit. Smoking status and alcohol use were retrieved using questionnaires. Laboratory parameters were assessed using routine laboratory techniques (Roche, Basel, Switzerland). The estimated glomerular filtration rate (eGFR) was calculated using the 2009 creatinine-based CKD-EPI formula.[36]

2.4. Statistical analyses

Data were analyzed using SPSS software version 23.0, R 3.5.2, and GraphPad Prism. Differences between two groups were assessed using independent sample t-tests, Mann-Whitney U tests, and Chi-squared tests. Differences between the three groups were assessed using one-way analysis of variance, Kruskal–Wallis tests, and Chi-squared tests.

Associations of iron deficiency and anemia with psychosocial symptoms and parameters of HRQoL were assessed using multinominal regression analyses for ordinal variables, logistic regression analyses for dichotomous variables, and linear regression analyses for continuous variables (with or without $\log_2$ transformation). After univariable analyses, analyses were adjusted for predefined potential confounders, including age, sex, eGFR, and time since transplantation in model 1, and additionally for diabetes, preemptive transplantation, living donor, history of rejection, C-reactive protein, and for variables that were significantly different between patients with and without iron deficiency, including calcineurin inhibitor use, angiotensin receptor blocker use, and angiotensin-converting enzyme inhibitor use in model 2. In model 3, we additionally adjusted for proton pump inhibitor use. Finally, anemia and/or iron deficiency were added to the models to assess whether the observed associations of iron deficiency or anemia were independent of each other.

Potential effect modification by age, sex, hemoglobin levels, and anemia was assessed by adding interaction terms to all presented regression models, but was not observed.

All models fulfilled the assumptions of linear regression. There were no missing data in any of the covariables included in the models. Results of linear regression are presented in standard deviation (SD) differences, which represent the standardized betas, indicating the number of SDs the dependent variable differs with the increase of one standard deviation in continuous variables or one unit increase for nominal variables. In all analyses, a two-sided P-value $< .05$ was considered statistically significant.

2.5. Sensitivity analyses

In sensitivity analyses, all linear regression analyses were repeated with hemoglobin on a continuous scale instead of anemia and with an alternative definition of iron deficiency (ie, ferritin levels < 300 μg/L and TSAT levels $< 20\%$) as previously used in KTRs,[32,33] instead of iron deficiency. In addition, subgroup analyses were performed after the exclusion of KTRs with iron supplementation and/or the use of erythropoietin-stimulating agents (ESAs).

3. Results

3.1. Baseline characteristics

A diagram visualizing the flow of participants through the study is provided in Figure 1. Participants excluded because of unmeasured iron status were included sooner posttransplant, more frequently used calcineurin inhibitors (CNIs), and less frequently used proton pump inhibitors (PPIs). In addition, subjects who were excluded for lacking HRQoL data more often used PPIs, less often underwent preemptive and living donor kidney transplantation, and had lower hemoglobin levels compared with included patients (Supplementary Table 2). In total, 814 KTRs (62% male, age 56 ± 13 years, 95% White) were included, at median age of 3 (interquartile range: 1-10) years after

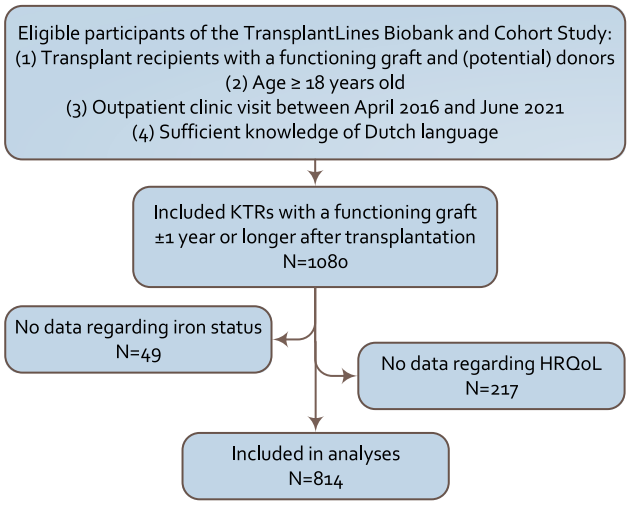

Figure 1. Patient flowchart visualizing patient selection from the TransplantLines Biobank and Cohort Study.
HRQoL, health-related quality of life; KTRs, kidney transplant recipients.

transplantation. In total, 229 (28%) KTRs had iron deficiency and 237 (29%) were anemic, among whom 84 (10%) had both iron deficiency and anemia (Fig. 2).

KTRs with iron deficiency were included more recently after transplantation compared to KTRs without iron deficiency (1 [1-7] vs 4 [1-11] years, $P < .001$). In addition, they more frequently had anemia (37% vs 26%, $P = .004$), diabetes (33% vs 25%, $P = .031$), and received a preemptive (45% vs 34%, $P = .007$) and living transplantation (62% vs 54%, $P = .034$). Moreover, KTRs with iron deficiency more frequently used CNIs (90% vs 80%, $P = .001$) and PPIs (79% vs 66%, $P < .001$), and less frequently used angiotensin receptor blockers (12% vs 18%, $P = .001$). Detailed baseline characteristics and PROs, stratified by iron status, are presented in the Table and Supplementary Table 3.

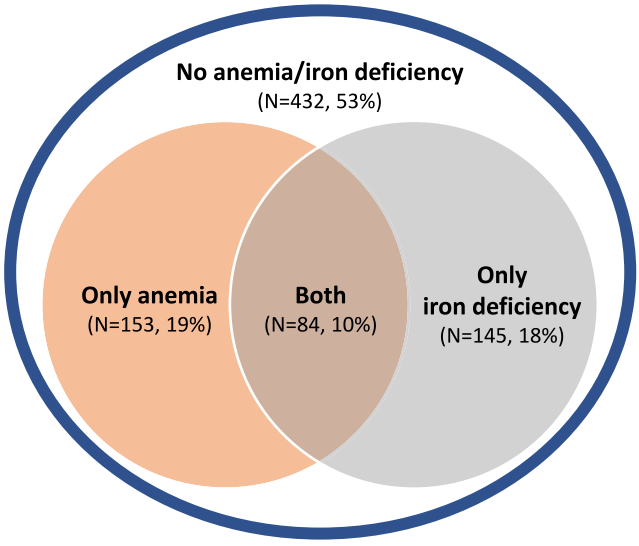

Figure 2. Venn diagram showing proportions of patients with anemia and/or iron deficiency.
Iron deficiency was defined as proposed by Kidney Disease: Improving Global Outcomes (KDIGO), the European Renal Best Practice Group, and the National Institute for Health and Care Excellence NG8 guidelines (ie, both transferrin saturation [TSAT] levels <20% and ferritin levels < 100 μg/L).[4-6] Anemia was defined as a hemoglobin level < 13 g/dL (for males) or <12 g/dL (for females) according to the World health Organization and KDIGO definitions.[2,3]

3.2. Association of iron deficiency and anemia with psychosocial symptoms

In total, 33% without iron deficiency and 35% with iron deficiency had mild to moderate fatigue, while 31% without iron deficiency and 35% with iron deficiency experienced severe fatigue. Additionally, 31% without iron deficiency and 38% with iron deficiency were at risk for sick leave/work disability; 17% without iron deficiency and 21% with iron deficiency had poor wellbeing; 23% without iron deficiency and 30% with iron deficiency reported anxiety symptoms; and 6% without iron deficiency and 12% without iron deficiency had major depressive symptoms. Furthermore, KTRs without iron deficiency had better concentration than those with iron deficiency (scores: 12 ± 6 vs 15 ± 8). In (multinominal) logistic regression analyses adjusted for age, sex, eGFR, time since transplantation, diabetes, preemptive transplantation, living donor, history of rejection, C-reactive protein, and use of CNIs, angiotensin receptor blockers, and angiotensin-converting enzyme inhibitors, iron deficiency was independently associated with an increased risk of severe fatigue (odds ratio $[OR]_{severe\ fatigue}$ 1.66, 95% confidence interval [CI] 1.10-2.51, $P = .016$), being at risk for sick leave or work disability (OR 1.62, 95% CI 1.15-2.29, $P = .006$), anxiety symptoms (OR 1.51, 95% CI 1.04-2.18, $P = .028$) and major depressive symptoms (OR 2.14, 95% CI 1.21-3.77, $P = .009$), as presented in model 2 in Supplementary Tables 4 and 5. To assess an association with concentration, we conducted linear regression analyses using concentration data on a continuous scale because of a lack of established cut-off scores. Analyses adjusted for the same variables revealed that iron deficiency was independently associated with a lower ability to concentrate (SD difference 0.27, 95% CI 0.11-0.42, $P < .001$), as presented in Supplementary Table 6. This table also shows the results of the previously reported outcomes on a continuous scale. After additional adjustment for PPI use and anemia, the associations of iron deficiency with severe fatigue, being at risk for sick leave or work disability, major depressive symptoms, and concentration remained materially unchanged, while associations with anxiety lost statistical significance ($P = .058$).

In contrast to iron deficiency, anemia was not associated with any of these outcomes, with point estimates markedly lower than iron deficiency ($P_{final\ model} > .7$ for all). No statistically significant association with poor wellbeing was observed for either iron deficiency or anemia, although point estimates for iron deficiency were numerically higher than those for anemia. The results of the fully adjusted models are presented in Figure 3.

3.3. Associations of iron deficiency and anemia with HRQoL

The mean physical and mental component scores of KTRs without iron deficiency and with iron deficiency were 70 ± 22

Table
Baseline characteristics according to strata of iron status.

Characteristics	No iron deficiency	Iron deficiency	*P*-value
Number of study subjects, *n* (%)	585 (72)	229 (28)	
Demographics			
Male sex, *n* (%)	358 (61)	143 (62)	.8
Age, y	56 ± 13	56 ± 14	.9
White, *n* (%)	558 (95)	221 (97)	.6
Body mass index, kg/m^2	27 ± 5	27 ± 4	.6
Educational level, *n* (%)			.6
Low	226 (39)	89 (39)	
Medium	199 (34)	84 (37)	
High	153 (27)	53 (24)	
Anemia, *n* (%)	153 (26)	84 (37)	.004
Diabetes, *n* (%)	146 (25)	75 (33)	.031
Time after transplantation, y	4 [1 to 11]	1 [1 to 7]	< .001
Preemptive transplantation, *n* (%)	199 (34)	102 (45)	.007
Living donor, *n* (%)	313 (54)	142 (62)	.034
History of rejection, *n* (%)	97 (17)	20 (9)	.006
Smoking status, *n* (%)			.5
Never smoked, *n (%)*	287 (49)	108 (47)	
Past smoker, *n* (%)	221 (38)	96 (42)	
Active smoker, *n (%)*	77 (13)	25 (11)	
Alcohol consumption, *n* (%)			.2
None	221 (38)	101 (44)	
< 7 units/wk	237 (41)	86 (38)	
< 7 units/wk	127 (22)	42 (18)	
Partnered, *n* (%)	460 (79)	177 (77)	.7
Employed*, *n* (%)	256 (59)	82 (50)	.077
Money shortage, *n* (%)	68 (13.1)	32 (14.9)	.6
Number of symptoms after Tx, *n*	6 [3 to 11]	7 [2 to 11]	.7
Laboratory blood levels measurements			
Hemoglobin, g/dL	13.6 ± 1.8	13.4 ± 1.8	.063
C-reactive protein, mg/L	1.8 [0.7 to 4.1]	2.2 [0.8 to 6.0]	.057
Plasma albumin, g/dL	4.4 ± 0.3	4.4 ± 0.3	.9
eGFR, mL/min/1.73m^2	51 ±18	55 ± 18	.011
Plasma iron, µmol/L	16 ± 5	9 ± 3	< .001
Ferritin, µg/L	129 [72 to 225]	33 [21 to 51]	< .001
Transferrin saturation, %	28 ± 9	13 ± 4	< .001
Medication use			
Prednisolone, *n* (%)	567 (97)	227 (99)	0.1
Calcineurin inhibitor, *n* (%)	465 (80)	205 (90)	.001

(*continued on next page*)

Table (*continued*)

Characteristics	No iron deficiency	Iron deficiency	*P*-value
Proliferation inhibitor, *n* (%)	507 (87)	201 (88)	0.8
mTOR inhibitor, *n* (%)	21 (4)	7 (3)	0.9
Iron supplements, *n* (%)	46 (8)	17 (7)	.054
Angiotensin receptor blockers, *n* (%)	103 (18)	27 (12)	.001
ACE inhibitors, *n* (%)	149 (25)	32 (14)	.9
Erythropoietin-stimulating agents, *n* (%)	14 (2)	4 (2)	.8
Proton pump inhibitors, *n* (%)	397 (66)	181 (79)	< .001

Data are presented as mean ± standard deviation, median [interquartile range], or number (valid%).
The significance of differences between groups is assessed using independent t-tests, Mann-Whitney U tests, and Chi Square tests, depending on the data distribution.
Data regarding educational level and money shortage are missing for 5 (0.6%) and 80 (10%) participants, respectively.
ACE, angiotensin-converting enzyme; eGFR, estimated glomerular filtration rate; mTOR, mammalian target of rapamycin.
Iron deficiency was defined as proposed by Kidney Disease: Improving Global Outcomes (KDIGO), the European Renal Best Practice Group, and the National Institute for Health and Care Excellence NG8 guidelines (ie, both transferrin saturation [TSAT] levels < 20% and ferritin levels < 100 μg/L).[4-6]
Anemia was defined as a hemoglobin level < 13 g/dL (for males) or < 12 g/dL (for females) according to the World health Organization and KDIGO definitions.[2,3]
* only assessed among nonretired participants (N = 602).

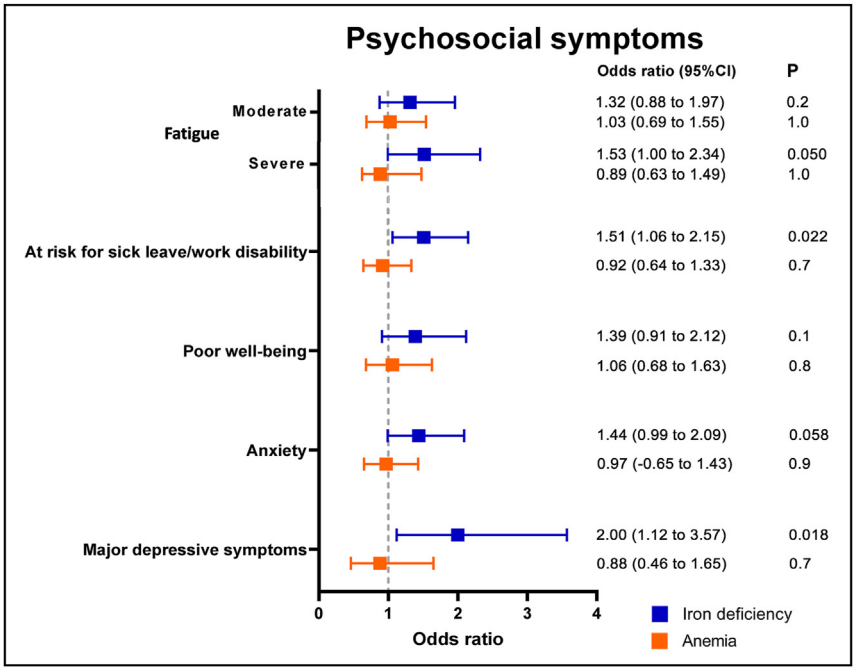

Figure 3. Associations of iron deficiency and anemia with psychosocial symptoms.
Results are adjusted for age, sex, estimated glomerular filtration rate and time since transplantation, diabetes, pre-emptive transplantation, living donor, history of rejection, C-reactive protein, calcineurin inhibitor, angiotensin receptor blocker, angiotensin-converting enzyme inhibitor use, proton pump inhibitors use, and iron deficiency (in case of anemia) or anemia (in case of iron deficiency). Abbreviations: CI, confidence interval. Iron deficiency was defined as proposed by Kidney Disease: Improving Global Outcomes (KDIGO), the European Renal Best Practice Group, and the National Institute for Health and Care Excellence NG8 guidelines (ie, both transferrin saturation [TSAT] levels < 20% and ferritin levels < 100 μg/L).[4-6] Anemia was defined as a hemoglobin level < 13 g/dL (for males) or < 12 g/dL (for females) according to World health Organization and KDIGO definitions.[2,3]

vs 67 ± 22 and 77 ± 18 vs 74 ± 19, respectively. Following a visual analog scale, KTRs without iron deficiency rated their health status higher than KTRs with iron deficiency (75 ± 14 vs 73 ± 16) on a scale from 0 to 100. The utility (EQ5D-3L) score was higher among those without iron deficiency compared with those with iron deficiency (0.87 ± 0.16 vs 0.85 ± 0.18), and both groups showed similar yet slightly higher utility scores compared with previous studies in kidney transplant recipients.[37] In linear regression analyses adjusted for potential confounders, iron deficiency was independently associated with lower physical component score (SD difference −0.21, 95% CI −0.35 to −0.06, $P = .006$), mental component score (SD difference −0.19, 95% CI −0.35 to −0.04, $P = .013$), health status assessed using a visual analog scale (SD difference −0.23, 95% CI −0.38 to −0.08, $P = .004$), and lower health status derived from the utility (EQ5D-3L) score (SD difference −0.20, 95% CI −0.36 to −0.05, $P = .010$). All associations remained materially unchanged after additional adjustment for PPIs and anemia. Again, no association of anemia with those outcomes were observed ($P = .4$, $P = 1.0$, $P = 0.6$, and $P = .2$ in the final model, respectively), as presented in Supplementary Table 7. To enhance understanding of the clinical significance of these effect sizes, the full model is detailed in Supplementary Table 8 and Figure 4, which allow for comparison of the association of iron deficiency on HRQoL relative to other factors. Notably, the magnitude of the association of iron deficiency with the HRQoL parameters is high compared with most other assessed associations.

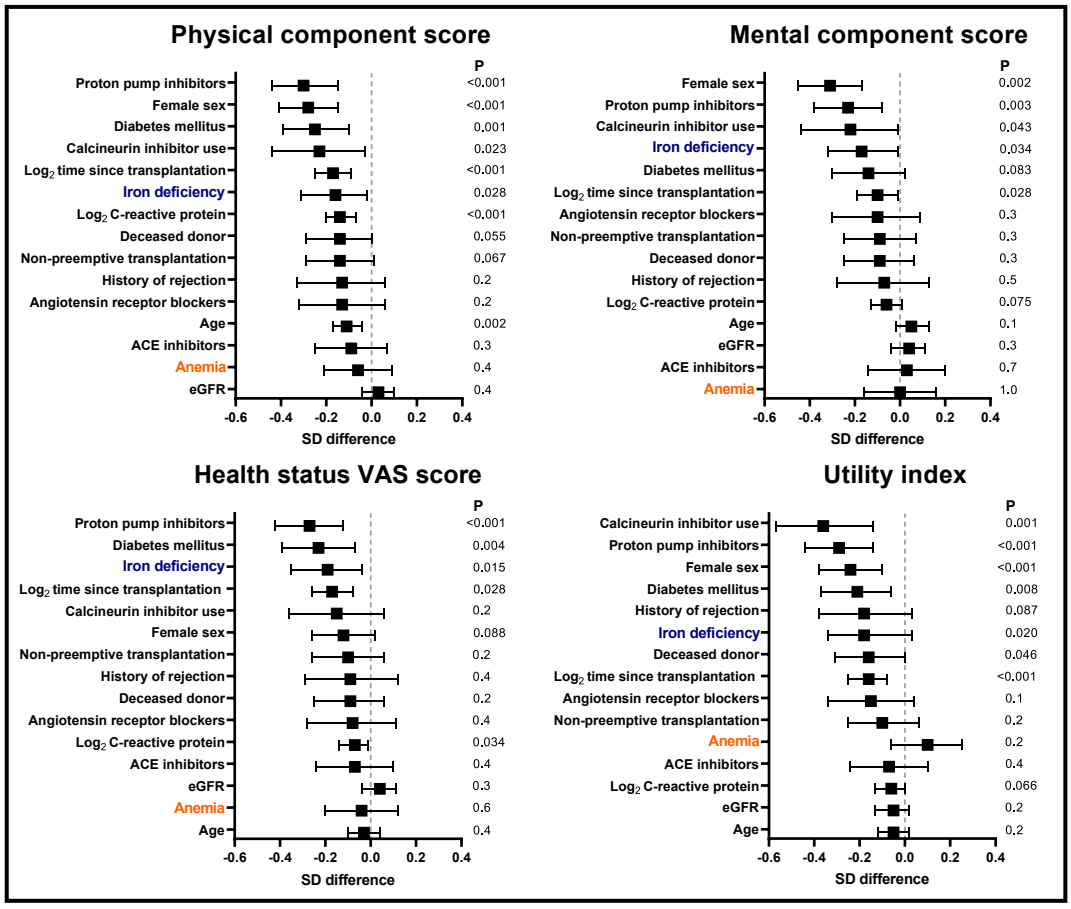

Figure 4. Final model of multivariable linear regression analyses with physical and mental component scores.
Results from linear regression are presented as the standard deviation (SD) difference, indicating how many SDs the dependent variables differ when anemia or iron deficiency is present. Results are adjusted for age, sex, estimated glomerular filtration rate and time since transplantation, diabetes, preemptive transplantation, living donor, history of rejection, C-reactive protein, calcineurin inhibitor, angiotensin receptor blocker, angiotensin-converting enzyme inhibitor use, proton pump inhibitor use, and iron deficiency (in case of anemia) or anemia (in case of iron deficiency). Iron deficiency was defined as proposed by Kidney Disease: Improving Global Outcomes (KDIGO), the European Renal Best Practice Group, and the National Institute for Health and Care Excellence NG8 guidelines (ie, both transferrin saturation [TSAT] level $< 20\%$ and ferritin level < 100 µg/L).[4-6] Anemia was defined as hemoglobin level < 13 g/dL (for males) or < 12 g/dL (for females) according to the World health Organization and KDIGO definitions.[2,3]. ACE, angiotensin-converting enzyme; eGFR, estimated glomerular filtration rate; VAS, visual analog scale.

Furthermore, we assessed associations with subdomains of HRQoL, derived from the SF-36 questionnaire. Here, iron deficiency was independently associated with lower general health (SD difference −0.24, 95% CI −0.39 to −0.08, $P = .002$), vitality (SD difference −0.17, 95% CI −0.32 to −0.01, $P = .035$), and emotional wellbeing (SD difference −0.25, 95% CI −0.41 to −0.09, $P = .002$) in the final model. Anemia was only associated with the subdomain general health (SD difference −0.16, 95% CI −0.32 to −0.01, $P = .039$), as presented in Supplementary Table 9.

3.4. Effect modification

No interaction of sex and age was present for the associations of iron deficiency with the assessed outcomes. In addition, there was no significant interaction of anemia or hemoglobin with these associations, further suggesting that the associations found with iron deficiency did not vary depending on anemia status or hemoglobin level.

3.5. Sensitivity analyses with hemoglobin levels

Sensitivity analyses with hemoglobin levels on a continuous scale instead of anemia yielded similar results compared to the primary analyses. Of all outcomes, hemoglobin was only significantly associated with physical component score in the final models adjusted for iron deficiency (SD difference 0.08, 95% CI 0.01 to 0.16, $P = .024$). Importantly, the associations between iron deficiency and outcomes remained materially unchanged after additional adjustment for hemoglobin instead of anemia (Supplementary Tables 10-12).

3.6. Sensitivity analyses with an alternative definition of iron deficiency

Sensitivity analyses with an alternative definition of iron deficiency also yielded results that were generally similar to the results of the primary analyses. In total, 284 (35%) KTRs met the criteria for the alternative iron deficiency definition. Similar to the

primary analyses, the alternative definition of iron deficiency was associated with the risk of sick leave or work disability, anxiety symptoms, major depressive symptoms, and health status assessed using a visual analog scale in the final model ($P_{\text{final model}} < .05$). The associations with all PROs generally remained similar using these alternative iron deficiency definitions compared with the primary analyses, but associations with anxiety, major depressive symptoms, and utility were notably stronger, while the associations with physical and mental component scores were numerically slightly weaker when applying the alternative definition (Supplementary Tables 10-12).

3.7. Sensitivity analyses in subgroups

In subgroup analyses after exclusion of KTRs with iron supplementation, iron deficiency was markedly more strongly associated with severe fatigue ($P = .012$), major depressive symptoms ($P = .002$), physical component score ($P = .009$), mental component score ($P = .014$), and impaired health status ($P = .036$). Most other associations remained generally comparable to primary analyses.

Similar results were obtained in analyses after the exclusion of KTRs with the use of ESAs, or both ESAs and iron (Supplementary Tables 13-15).

4. Discussion

This study shows that iron deficiency but not anemia was associated with more fatigue, worse concentration, more anxiety, higher risks of major depressive symptoms and sick leave, and lower physical and mental component scores of HRQoL in models including both conditions. In the fully adjusted models, iron deficiency was associated with an estimated 53% higher risk of severe fatigue, a 100% higher risk of major depressive symptoms, and a 51% higher chance of being at risk for sick leave/work disability. These associations were generally stronger in sensitivity analyses with the exclusion of patients on iron supplementation. Associations were generally slightly weaker using an alternative definition of iron deficiency, which supports the definition proposed in current guidelines that was used in the primary analyses (ie, both TSAT levels $< 20\%$ and ferritin levels < 100 µg/L).

Life after kidney transplantation keeps growing in importance as a result of the increasing numbers of patients living with a kidney graft. Although increasing scientific attention is being paid to the subject, psychosocial symptoms, including fatigue, anxiety, and depressive symptoms remain highly prevalent in KTRs.[2] Moreover, HRQoL in KTRs remains impaired compared with the general population.[2,3] There are no targeted therapies to improve these PROs. Considering the key roles that iron plays in muscle (eg, for myoglobin production and mitochondrial function), brain (eg, for myelin formation and neurotransmitter synthesis), and cardiovascular health (eg, for the contractility of cardiomyocytes)[9-11], and the high prevalence of iron deficiency in KTRs,[8] we hypothesized that iron status is a promising target to improve PROs and HRQoL.

Thus far, epidemiological support for this notion has been lacking in KTRs. The current study shows that iron deficiency is robustly associated with more fatigue, worse concentration, more anxiety, higher risks of major depressive symptoms and sick leave, and lower physical and mental component scores of HRQoL in KTRs. These observations put forward iron deficiency, independent of anemia, as a promising potential modifiable target to improve PROs in KTRs. Notably, the associations of iron deficiency with psychosocial symptoms and lower HRQoL remained, whereas the associations of anemia with these parameters were abrogated in a model encompassing both conditions. Effect modification analyses showed no interaction of hemoglobin on the associations, further supporting that iron deficiency is associated with psychosocial symptoms and lower HRQoL regardless of hemoglobin level.

Symptoms can result in functional limitations and lower HRQoL.[38] First, we assessed whether iron deficiency is associated with psychosocial symptoms. The independent, consistent associations of iron deficiency with more fatigue, worse concentration, more anxiety, higher risks of major depressive symptoms, and sick leave are novel, yet in line with previous studies supporting the multiple effects that iron deficiency may have on mental and physical symptoms.[39,40] The observational design of this study does not allow for drawing causal conclusions. However, the potential importance of the associations of iron deficiency with psychosocial symptoms is further supported by the consistent associations of iron deficiency with physical and mental component scores of HRQoL.

The associations of iron deficiency with lower HRQoL are in line with observations in other populations, such as in patients with heart failure.[41] The associations of iron deficiency with lower HRQoL identified in patients with heart failure were the reason for subsequent randomized clinical trials, which confirmed rapid yet long-lasting improvements in HRQoL after treatment with intravenous ferric carboxymaltose.[42-44] Similar beneficial treatment effects of iron deficiency correction on HRQoL were observed in a trial among heart transplant recipients.[45] Importantly, the improvements in HRQoL observed in these trials were independent of the presence of concomitant anemia.[43-45] In addition, a large proportion of the participants in these trials had chronic kidney disease, which appeared not to adversely affect the benefit of intravenous iron supplementation on HRQoL. These findings, combined with observations from our current study, raise the suggestion that correction of iron deficiency, regardless of hemoglobin levels, may improve PROs and HRQoL in KTRs. Clearly, this hypothesis will have to be tested in interventional studies, as conclusions about causality cannot be drawn from the current observational study. Other observational study designs, such as studies applying, eg, propensity scores, may be considered in the future. However, ultimately, we need clinical trials to investigate the effect of iron deficiency correction on PROs and HRQoL in KTRs.

In current nephrological practice, iron status is primarily assessed in the context of coexisting anemia. Exemplary of this current practice is that, in the United States, iron status is assessed in 53% of patients with relatively low hemoglobin levels (group mean: 9.1 g/dL), compared to iron status assessment in only 14% of patients with relatively high hemoglobin levels (group mean: 13.4 g/dL).[12] Our study results suggest that clinicians may also consider assessing iron deficiency, regardless of coexisting anemia, particularly in the context of improving psychosocial symptoms and HRQoL in KTRs. This hypothesis may be particularly relevant in KTRs, given their altered iron homeostasis.[8] Indeed, next to sex, use of CNIs, and diabetes, the association of iron deficiency with physical and mental component scores of HRQoL showed the largest magnitude among all assessed independent variables. This notion, alongside the mere magnitude of the associations with iron deficiency, highlights the clinical relevance of iron deficiency and the hypothetical effect size of iron deficiency correction.

The major strength of this study is the unique availability of clinical and psychometric data in a large population of KTRs. This availability allows for the identification of associations between clinical parameters and PROs, which may help identify potential targets to improve HRQoL. Thus far, this topic remains underrepresented in the transplant literature, and there is a lack of targets to improve PROs. The results of the current study are encouraging and put forward iron status as a potential target for improvement. Moreover, the extensive availability of phenotypical data allowed us to adjust for potential confounders. In addition, extensive sensitivity analyses were performed, in which the association of iron deficiency with HRQoL was robust and independent of hemoglobin level. Additionally, the magnitudes of the association between iron deficiency and HRQoL were larger and larger after the exclusion of KTRs with iron supplementation and/or the use of ESAs, in line with the hypothesis.

We also acknowledge several study limitations. The study population was predominantly White, derived from a single European center, which calls for prudence to extrapolate these results to other populations. Moreover, patients with iron deficiency differed from those without iron deficiency. Although the observed associations between iron deficiency and the PROs remained fundamentally unchanged upon adjustment for the variables that differed significantly at baseline, residual confounding cannot be excluded. Moreover, a proportion of the population had to be excluded, mainly because of the unavailability of data on HRQoL. Indeed, an imperfect response rate is inherent to PRO data in large cohorts.[46-48] The obtained response rate of 79% is excellent compared with other studies on PROs.[46-48] However, the excluded population differed from the included population in certain aspects. We adjusted for the variables that differed between included and excluded subjects in all regression analyses, which did not materially change the results. While this is reassuring regarding the validity of our findings, selection bias may still be present. Moreover, despite extensive adjustments and sensitivity analyses, no causality can be attributed to the current study because of the observational, cross-sectional study design. Finally, although iron deficiency was consistently associated with worse PROs, intervention studies are needed to confirm that the observed differences are of such magnitude that patients experience improvements upon iron deficiency correction.

In conclusion, the current study shows the potential clinical importance of iron deficiency for PROs, including HRQoL in KTRs, independent of hemoglobin levels or the presence of anemia. The observed effect sizes for the associations of iron deficiency with PROs evoke interest and shed light on the potential impact that iron deficiency may have on KTRs, regardless of coexisting anemia. These results pave the way for interventional studies investigating the effects of iron deficiency correction with iron therapy on PROs, including HRQoL in KTRs.

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Author contributions

Data acquisition: D. Kremer, T. J. Knobbe, S.J.L. Bakker, and M. F. Eisenga; data analyses: D. Kremer, T. J. Knobbe, and M. F. Eisenga; data interpretation: all authors (D. Kremer, T. J. Knobbe, J.S.J. Vinke, D. Groothof, A. Post, C. Annema, A. C. Abrahams, B. C. van Jaarsveld, M.H. de Borst, S. P. Berger, TransplantLines Investigators, S.J.L. Bakker, and M. F. Eisenga); cohort design and supervision: S.J.L. Bakker and M. F. Eisenga. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

Data availability

Public sharing of individual participant data was not included in the informed consent forms of the TransplantLines Biobank and Cohort Study, but data will be made available to interested researchers upon reasonable request after approval by the TransplantLines scientific committee.

Declaration of competing interest

The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. M.F. Eisenga has declared receiving consultant fees from Vifor

Pharma and Cablon Medical; serving on the Advisory Board for Cablon Medical and GlaxoSmithKline; and receiving speaker fees from Vifor Pharma, Pharmacosmos, and Astellas. All other authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2024.03.017.

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References

1. Hariharan S, Israni AK, Danovitch G. Long-term survival after kidney transplantation. *N Engl J Med*. 2021;385(8):729–743. https://doi.org/10.1056/nejmra2014530.
2. van Sandwijk MS, Al Arashi D, van de Hare FM, et al. Fatigue, anxiety, depression and quality of life in kidney transplant recipients, haemodialysis patients, patients with a haematological malignancy and healthy controls. *Nephrol Dial Transplant*. 2019;34(5):833–838. https://doi.org/10.1093/ndt/gfy103.
3. Wang Y, Hemmelder MH, Bos WJW, et al. Mapping health-related quality of life after kidney transplantation by group comparisons: a systematic review. *Nephrol Dial Transplant*. 2021;36(12):2327–2339. https://doi.org/10.1093/ndt/gfab232.
4. Kawada N, Moriyama T, Ichimaru N, et al. Negative effects of anemia on quality of life and its improvement by complete correction of anemia by administration of recombinant human erythropoietin in posttransplant patients. *Clin Exp Nephrol*. 2009;13(4):355–360. https://doi.org/10.1007/s10157-009-0170-x/figures/5.
5. Babitt JL, Eisenga MF, Haase VH, et al. Controversies in optimal anemia management: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Conference. *Kidney Int*. 2021;99(6):1280–1295. https://doi.org/10.1016/j.kint.2021.03.020.
6. Gafter-Gvili A, Ayalon-Dangur I, Cooper L, et al. Posttransplantation anemia in kidney transplant recipients: A retrospective cohort study. *Medicine (Baltimore)*. 2017;96(32):e7735. https://doi.org/10.1097/md.0000000000007735.
7. Choukroun G, Kamar N, Dussol B, et al. CAPRIT study Investigators. Correction of postkidney transplant anemia reduces progression of allograft nephropathy. *J Am Soc Nephrol*. 2012 Feb;23(2):360–368. https://doi.org/10.1681/asn.2011060546.
8. Vinke JSJ, Francke MI, Eisenga MF, Hesselink DA, de Borst MH. Iron deficiency after kidney transplantation. *Nephrol Dial Transplant*. 2021;36(11):1976–1985. https://doi.org/10.1093/ndt/gfaa123.
9. Vinke JSJ, Gorter AR, Eisenga MF, et al. Iron deficiency is related to lower muscle mass in community-dwelling individuals and impairs myoblast proliferation. *J Cachexia Sarcopenia Muscle*. 2023;14(4):1865–1879. https://doi.org/10.1002/jcsm.13277.
10. Stugiewicz M, Tkaczyszyn M, Kasztura M, Banasiak W, Ponikowski P, Jankowska EA. The influence of iron deficiency on the functioning of skeletal muscles: experimental evidence and clinical implications. *Eur J Heart Fail*. 2016;18(7):762–773. https://doi.org/10.1002/ejhf.467.
11. Von Haehling S, Jankowska EA, Van Veldhuisen DJ, Ponikowski P, Anker SD. Iron deficiency and cardiovascular disease. *Nat Rev Cardiol*. 2015;12(11):659–669. https://doi.org/10.1038/nrcardio.2015.109.
12. 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(4):613–624. https://doi.org/10.1093/ckj/sfz091.
13. Grimmelt AC, Cohen CD, Fehr T, Serra AL, Wüthrich RP. Safety and tolerability of ferric carboxymaltose (FCM) for treatment of iron deficiency in patients with chronic kidney disease and in kidney transplant recipients. *Clin Nephrol*. 2009;71(2):125–129. https://doi.org/10.5414/cnp71125.
14. Rozen-Zvi B, Gafter-Gvili A, Zingerman B, et al. Intravenous iron supplementation after kidney transplantation. *Clin Transplant*. 2012;26(4):608–614. https://doi.org/10.1111/j.1399-0012.2012.01602.x.
15. Mudge DW, Tan KS, Miles R, et al. A randomized controlled trial of intravenous or oral iron for posttransplant anemia in kidney transplantation. *Transplantation*. 2012;93(8):822–826. https://doi.org/10.1097/tp.0b013e318248375a.
16. Kliger AS, Foley RN, Goldfarb DS, et al. KDOQI us commentary on the 2012 KDIGO clinical practice guideline for anemia in CKD. *Am J Kidney Dis*. 2013;62(5):849–859. https://doi.org/10.1053/j.ajkd.2013.06.008.
17. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *PLoS Med*. 2007;4(10):e296. https://doi.org/10.1371/journal.pmed.0040296.
18. Eisenga MF, Gomes-Neto AW, Van Londen M, et al. Rationale and design of TransplantLines: a prospective cohort study and biobank of solid organ transplant recipients. *BMJ Open*. 2018;8(12):1–13. https://doi.org/10.1136/bmjopen-2018-024502.
19. Vercoulen JHMM, Alberts M, Bleijenberg G. De Checklist Individual Strength (CIS). *Gedragstherapie*. 1999;32:131–136.
20. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. *J Psychosom Res*. 1994;38(5):383–392. https://doi.org/10.1016/0022-3999(94)90099-x.
21. Bultmann U, Beurskens AJHM, Bleijenberg G, Vercoulen JHMM, Kant U. Measurement of prolonged fatigue in the working population: determination of a cutoff point for the checklist individual strength. *J Occup Health Psychol*. 2000;5(4):411–416. https://doi.org/10.1037/1076-b998.5.4.411.
22. Topp CW, Østergaard SD, Søndergaard S, Bech P. The WHO-5 well-being index: a systematic review of the literature. *Psychother Psychosom*. 2015;84(3):167–176. https://doi.org/10.1159/000376585.
23. Julian LJ. Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). *Arthritis Care Res (Hoboken)*. 2011;63:S467–S472. https://doi.org/10.1002/acr.20561. Suppl 11(0 11).
24. Kroenke K, Spitzer RL, Williams JBW. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001;16(9):606–613. https://doi.org/10.1046/j.1525-1497.2001.016009606.x.
25. Aaronson NK, Muller M, Cohen PD, et al. Translation, validation, and norming of the dutch language version of the SF-36 Health Survey in community and chronic disease populations. *J Clin Epidemiol*. 1998;51(11):1055–1068. https://doi.org/10.1016/s0895-4356(98)00097-3.
26. EQ-5D User Guides – EQ-5D. Accessed October 18, 2023. https://euroqol.org/publications/user-guides/.

27. Cleemput I, Kesteloot K, Moons P, et al. The construct and concurrent validity of the EQ-5D in a renal transplant population. *Value Health*. 2004;7(4):499–509. https://doi.org/10.1111/j.1524-4733.2004.74013.x.
28. Gomez-Besteiro MI, Santiago-Pérez MI, Alonso-Hernández Á, Valdés-Cañedo F, Rebollo-Álvarez P. Validity and reliability of the SF-36 questionnaire in patients on the waiting list for a kidney transplant and transplant patients. *Am J Nephrol*. 2004;24(3):346–351. https://doi.org/10.1159/000079053.
29. Lamers LM, McDonnell J, Stalmeier PF, Krabbe PF, Busschbach JJ. The Dutch tariff: results and arguments for an effective design for national EQ-5D valuation studies. *Health Econ*. 2006;15(10):1121–1132. https://doi.org/10.1002/hec.1124.
30. Heemann U, Abramowicz D, Spasovski G, Vanholder R. Endorsement of the Kidney Disease Improving Global Outcomes (KDIGO) guidelines on kidney transplantation: a European Renal Best Practice (ERBP) position statement. *Nephrol Dial Transplant*. 2011;26(7):2099–2106. https://doi.org/10.1093/ndt/gfr169.
31. Ratcliffe LEK, Thomas W, Glen J, et al. Diagnosis and management of iron deficiency in CKD: a summary of the NICE guideline recommendations and their rationale. *Am J Kidney Dis*. 2016;67(4):548–558. https://doi.org/10.1053/j.ajkd.2015.11.012.
32. Eisenga MF, Van Londen M, Leaf DE, et al. C-terminal fibroblast growth factor 23, iron deficiency, and mortality in renal transplant recipients. *Am Soc Nephrol*. 2017;28(12):3639–3646. https://doi.org/10.1681/asn.2016121350.
33. Eisenga MF, Minović I, Berger SP, et al. Iron deficiency, anemia, and mortality in renal transplant recipients. *Transplant International*. 2016;29(11):1176–1183. https://doi.org/10.1111/tri.12821.
34. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. https://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf. Accessed 1 March 2024.
35. Drüeke TB, Parfrey PS. Summary of the KDIGO guideline on anemia and comment: reading between the (guide)line(s). *Kidney Int*. 2012;82(9):952–960. https://doi.org/10.1038/ki.2012.270.
36. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604–612. https://doi.org/10.7326/0003-4819-150-9-200905050-00006.
37. Wyld M, Morton RL, Hayen A, Howard K, Webster AC. A systematic review and meta-analysis of utility-based quality of life in chronic kidney disease treatments. *PLoS Med*. 2012;9(9). https://doi.org/10.1371/journal.pmed.1001307.
38. Wilson IB, Cleary PD. Linking clinical variables with health-related quality of life. A conceptual model of patient outcomes. *JAMA*. 1995;273(1):59–65.
39. Ciulei MA, Ahluwalia N, McCormick BJJ, Teti DM, Murray-Kolb LE. Iron deficiency is related to depressive symptoms in United States nonpregnant women of reproductive age: a cross-sectional analysis of NHANES 2005-2010. *J Nutr*. 2023;153(12):3521–3528. https://doi.org/10.1016/j.tjnut.2023.09.023.
40. Vinke JSJ, Ziengs AL, Buunk AM, et al. Iron deficiency and cognitive functioning in kidney transplant recipients: findings of the TransplantLines biobank and cohort study. *Nephrol Dial Transplant*. 2023;38(7):1719–1728. https://doi.org/10.1093/ndt/gfad013.
41. Comín-Colet J, Enjuanes C, González G, et al. Iron deficiency is a key determinant of health-related quality of life in patients with chronic heart failure regardless of anaemia status. *Eur J Heart Fail*. 2013;15(10):1164–1172. https://doi.org/10.1093/eurjhf/hft083.
42. Comin-Colet J, Lainscak M, Dickstein K, et al. The effect of intravenous ferric carboxymaltose on health-related quality of life in patients with chronic heart failure and iron deficiency: a subanalysis of the FAIR-HF study. *Eur Heart J*. 2013;34(1):30–38. https://doi.org/10.1093/eurheartj/ehr504.
43. 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(25):2436–2448. https://doi.org/10.1056/nejmoa0908355.
44. Jankowska EA, Kirwan BA, Kosiborod M, et al. The effect of intravenous ferric carboxymaltose on health-related quality of life in iron-deficient patients with acute heart failure: the results of the AFFIRM-AHF study. *Eur Heart J*. 2021;42(31):3011–3020. https://doi.org/10.1093/eurheartj/ehab234.
45. Englund KB, Oestby CM, Rolid K, et al. Health related quality of life in iron deficient heart transplant recipients receiving intravenous iron supplement: a prespecified secondary endpoint in the IronIC trial. *J Heart Lung Transplant*. 2021;40(4):S281–S282. https://doi.org/10.1016/j.healun.2021.01.800.
46. Coste J, Quinquis L, Audureau E, Pouchot J. Non response, incomplete and inconsistent responses to self-administered health-related quality of life measures in the general population: Patterns, determinants and impact on the validity of estimates - a population-based study in France using the MOS SF-36. *Health Qual Life Outcomes*. 2013;11(1):1–15. https://doi.org/10.1186/1477-7525-11-44/tables/4.
47. Ruseckaite R, Mudunna C, Caruso M, Ahern S. Response rates in clinical quality registries and databases that collect patient reported outcome measures: a scoping review. *Health Qual Life Outcomes*. 2023;21(1):1–19. https://doi.org/10.1186/s12955-023-02155-5.
48. Peters M, Crocker H, Jenkinson C, Doll H, Fitzpatrick R. The routine collection of patient-reported outcome measures (PROMs) for long-term conditions in primary care: a cohort survey. *BMJ Open*. 2014;4(2):e003968. https://doi.org/10.1136/bmjopen-2013-003968.