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ORIGINAL ARTICLE

Association between malnutrition–inflammation score and risk of subsequent self-reported bone fractures in prevalent kidney transplant recipients

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Abstract

Summary Chronic inflammation and protein energy wasting (PEW) syndrome are common in kidney transplant recipients (KTR). The presence of inflammation and PEW syndrome can directly affect bone resorption and bone formation, leading to bone loss and fractures. We showed PEW is independently associated with new clinically detected bone fractures in prevalent KTR.

Introduction Kidney transplant recipients (KTR) have a 4-fold higher risk of fracture compared to the general population. Chronic inflammation and PEW syndrome are common in KTR and are associated with poor outcomes. We hypothesized that the Malnutrition–Inflammation Score (MIS), a validated measure of PEW, is associated with higher risk of bone fractures in KTR.

Methods This prospective cohort study included 839 prevalent KTR from a Central European academic center. MIS, a semi-quantitative instrument of PEW, was calculated at the study entry. Self-reported history of fractures was recorded during the 2-year follow-up period. The association between MIS and bone fractures was examined in logistic regression analyses with adjustment for age, gender, eGFR, smoking habits, history of pre-transplant bone fractures, and acute rejection.

Results Mean age was 51 ± 13 years, and 56% of patients were males with median (interquartile range) transplant vintage 69 (38–112) months, estimated glomerular filtration rate 55 ± 21 ml/min/1.73 m^2, and calculated MIS 3 (2–4) at enrollment. Fifty-five (7%) patients experienced self-reported bone fractures during the 2-year follow-up period. Higher MIS score showed linear association with increased risk of fracture. Each one-point higher MIS was associated with 23% higher risk of bone fractures (odds ratio (OR) and 95% CI 1.23, 1.12–1.34), which remained significant after multivariable adjustments (OR 1.17, 95% CI 1.06–1.29).

Conclusion The MIS is independently associated with new clinically detected bone fractures in prevalent KTR.

Keywords Bone fracture · Kidney transplant · Malnutrition–inflammation score · Protein energy wasting

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Introduction

Kidney transplant recipients (KTR) are at a higher risk of mineral–bone disorders and bone fractures compared to the general population [1, 2]. The incidence of post-transplant bone fractures is approximately 3%, and this incidence might further increase with decline in kidney function [3, 4]. Post-transplant bone fractures are associated with 5-fold higher incidence rate of hospitalization, 60% higher risk of mortality, and reduced quality of life [5, 6]. Although several pre- and post-transplant risk factors such as older age, female gender, underweight, presence of diabetes, history of previous fracture, smoking habits, longer time on dialysis, cumulative dose of corticosteroids, vitamin D deficiency, elevated intact parathyroid hormone (iPTH), and elevated serum fibroblast growth factor 23 (FGF-23) have been identified to play a role in bone fractures, additional risk factors may be present in this high risk population.

The presence of chronic inflammation and protein energy wasting (PEW) syndrome, also previously known as Malnutrition–Inflammation Complex Syndrome (MICS), is common in the chronic kidney disease (CKD) population as well as in KTR [7, 8]. Patients with PEW have higher risk of cardiovascular disease, increased mortality, and poor outcomes [9]. The severity of PEW/MICS can be evaluated using a semiquantitative tool, the Malnutrition–Inflammation Score (MIS), which was previously designed for dialysis patients and further validated for use in KTR [10, 11]. Our previous studies demonstrated that MIS is a useful and simple instrument, which is associated with increased risk of mortality, graft loss, anemia, and depressive symptoms in KTR [12–14]; on the other hand, the relationship between MIS and incident bone fractures remains insufficiently explored.

Various pathophysiologic mechanisms of cytokine-induced bone loss have been proposed in patients with inflammatory disorders, and similar pathways may also play role in KTR with PEW [15, 16]. PEW may contribute to increased catabolism, activation of proinflammatory cytokines, and loss of lean body mass; consequently, this can directly stimulate bone resorption and reduce bone formation, leading to bone loss and fractures [15, 16]. Therefore, PEW may play a direct key role in the development of bone fractures among other traditional risk factors and the MIS score may play additional role in the pathophysiology of bone fracture in KTR.

To the best of our knowledge, it is unclear whether higher MIS is associated with increased risk of incident bone fracture in KTR. To address this knowledge gap, we examined the association between MIS and the risk of bone fractures in prevalent KTR. We hypothesized that a higher MIS score would be associated with higher risk of bone fracture.

Methods

Study design and participants

In this prospective cohort study (Malnutrition–Inflammation in Transplant—Hungary Study [MINIT-HU Study]), all adult patients (≥ 18 years of age, $n = 1214$) were enrolled at a single kidney transplant center at the Department of Transplantation and Surgery at Semmelweis University, Budapest, during the inclusion period of February, 2007 to August 2007 [13, 17, 18]. Baseline characteristics were defined at the time of cohort inclusion. Patients who experienced acute rejection within the last 4 weeks prior to study entry received kidney transplantation in the previous 3 months, had active hospitalization, or had acute infection and/or bleeding ($n = 16$), and patients who refused to participate ($n = 205$) in this study were excluded. Patients who had missing fracture records ($n = 151$) and missing MIS scores ($n = 3$) also were excluded from this study. The algorithm for the cohort definition is shown in Fig. 1, and the final study population consisted of 839 patients.

The following demographic data and details of medical history were collected at baseline: age, gender, history of hypertension, coronary heart disease, diabetes, smoking history, previous (pre-transplant) history of bone fractures, co-morbidities (the modified Charlson Comorbidity Index), body mass index (BMI), abdominal circumference, transplantation-related data with immunosuppressant medications, and details of medications used to manage bone–mineral disease. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [19]. The study was approved by the Institutional Ethics Committee (49/2006 and 14-03481-XM) of the Semmelweis University and the University of Tennessee, and all patients provided written and verbal informed consent before enrollment. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism." Patients were examined according to good medical and laboratory practice and in keeping with the recommendations set forth by the Declaration of Helsinki Guidelines for Biomedical Research Involving Human Subjects.

Outcome variable: post-transplant bone fractures

The number of fractures and their detailed localizations (upper limb, lower limb/hip, spine, rib, pelvis, and others) were recorded during the 2-year follow-up period. The information was obtained from patients (based on questionnaire assessing the location and the number of fractures) as well as from the patients' medical chart yearly at the time of the follow-up visit. We recorded self-reported bone fractures only, if this was a

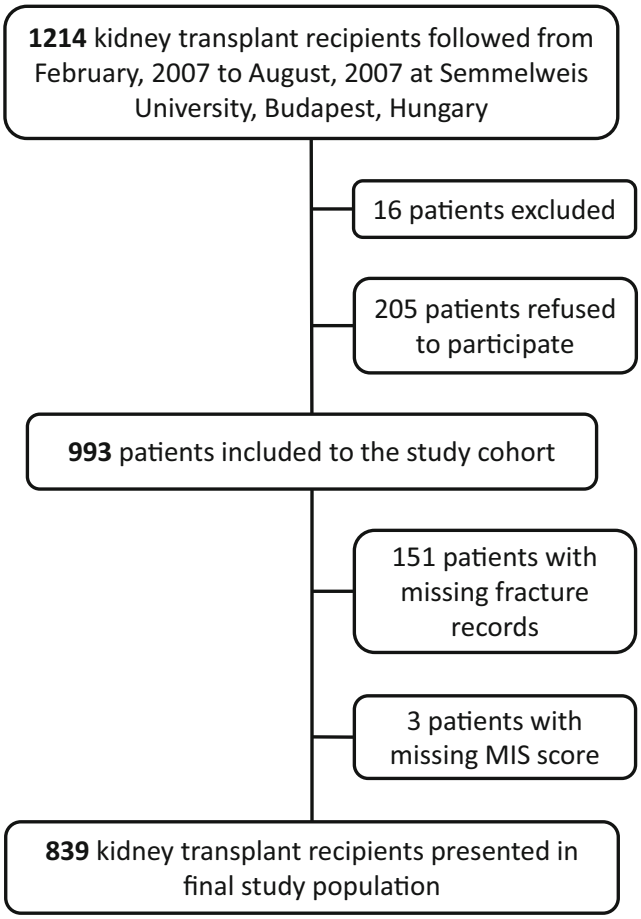

Fig. 1 Flowchart of the selection of patients

physician confirmed bone fracture or there was a corresponding radiology report in the Center's medical records.

Exposure variable: malnutrition–inflammation score

The MIS has 10 components, each with four levels of severity, from 0 (normal) to 3 (severely abnormal) [10]. The sum of all 10 MIS components ranges from 0 (normal) to 30 (severely malnourished); a higher score reflects a more severe degree of malnutrition and inflammation status. In contrast to the original MIS, we did not include dialysis vintage in the score in this analysis; however, co-morbidity was computed as follows: 0—no medical illnesses except CKD; 1—mild co-morbidity, excluding major co-morbid conditions (MCCs) such as congestive heart failure classes III–IV, severe coronary artery disease, clinically evident acquired immunodeficiency syndrome, moderate to severe chronic obstructive pulmonary disease and metastatic malignancies; score 2—moderate co-morbidity (including one of the diseases listed under MCCs); and score 3—two or more MCCs. All subjective global assessment (SGA) was performed by a physician according to conventional SGA guidelines [20]. This score has been validated in KTR. [12]

Confounder variables: laboratory data and comorbidities

Comorbidity was assessed using the modified Charlson co-morbidity index [21]. History of previous bone fractures (before to enter the study) was obtained from patients same way as described above. All routine biochemical laboratory data were collected and measured at the baseline visit including bone–mineral markers (serum phosphorus, total calcium, iPTH, alkaline phosphatase, osteoprotegerin, osteocalcin, FGF-23, and 25-OH vitamin D levels) and inflammatory-nutritional markers (high sensitive C-reactive protein (hsCRP), interleukin 6 (IL6), tumor necrosis factor alpha (TNF-α), and serum albumin).

Confounder variables: transplant-related data

Transplant-related data was collected from medical records and included information about immunosuppressant treatment regimen, transplant vintage (i.e., time elapsed since the time of the transplantation), previous time on dialysis, total end-stage renal disease (ESRD) time, donor type (live or deceased), donor age, cold ischemic time, history of delayed graft function, and history of acute rejection.

Confounder variables: immunosuppressive therapy

Standard immunosuppressive therapy included prednisolone, with either cyclosporine A (CsA) microemulsion formulation (Neoral) or tacrolimus, combined with mycophenolate mofetil (MMF) or azathioprine or sirolimus. Our center does not practice "steroid free" or "early steroid withdrawal" transplantation, and the maintenance prednisolone 5 mg daily dose is reached by the end of 4 weeks, and all patients remain on this dose indefinitely.

Statistical analysis

All data are summarized as percentages for categorical variables and as mean ± standard deviation (SD) or median (interquartile range) for continuous variables, as appropriate. Descriptive data are presented according to the two MIS groups using cutoff point 5 (lowest: MIS < 5 and highest: MIS ≥ 5) based on a meta-analysis of contemporary cohort studies from the International Society of Renal Nutrition and Metabolism (ISRNM) [22]. Categorical variables were compared using χ^2 tests, and continuous variables were compared using t tests or Mann–Whitney U tests, as appropriate.

The association between MIS as continuous variable and overall risk of incident bone fractures was examined using unadjusted and adjusted logistic regression analyses. We also performed ordinal logistic regression analysis to examine the

association between MIS and the number of fractures (categorized as one, two, and three or more fractures during the follow-up period). The following potential confounders were included in the multivariable adjusted model based on theoretical considerations based on previous results from the literature: age, gender, eGFR, smoking status, history of pre-study bone fractures, and acute rejection to account for a larger steroid exposure. Nonlinear associations between MIS score and bone fracture were assessed using cubic splines.

We performed several sensitivity analyses to evaluate the robustness of our main findings. First, we used propensity score (PS) adjustment in lieu of adjusting our regression models for individual confounders. As we had limited number of events, therefore limited possibility to adjust for confounders in our main model, we calculated the propensity score of having low (< 5) and high (≥ 5) MIS score in order to incorporate information from multiple confounding factors in a single variable. We calculated the PS in a logistic regression model using 24 variables that satisfied balancing criteria (Supplement Table 1).

Second, the associations between risk of bone fractures (both overall and ordered by severity) and MIS score were also examined using logistic and ordinal logistic regression analyses in selected subgroups of patients stratified by age (< 45 or ≥ 45 years), eGFR (< 60 or ≥ 60 ml/min/1.73 m^2), total ESRD time (< 9 or ≥ 9 years), iPTH (< 150 or ≥ 150 pg/ml), history of pre-transplant bone fractures, smoking habits, and acute rejection. Potential interactions were formally tested by including relevant interaction terms to assess effect modification.

P values are two-sided and reported as significant at < 0.05 for all analyses. All analyses were conducted using STATA MP version 15 (STATA Corporation, College Station, TX).

Results

Baseline characteristics

Baseline characteristics of the overall cohort and in patients stratified by MIS level are presented in Table 1. The mean ± SD age of the study population was 51 ± 13 years (range 18–80 years), and 56% of participants were male. Of the cohort, 96% of recipients received a kidney from a deceased donor and 34% and 26% of recipients experienced acute rejection and delayed graft function, respectively. The median previous time on dialysis and median time since last kidney transplantation was 20 months (interquartile range (IQR) 9–38 months) and 69 months (IQR 38–112 months), respectively.

Patients with higher MIS were more likely to have coronary heart disease, diabetes, history of pre-transplant bone fractures, lower eGFR and BMI, decreased serum levels of 25-OH vitamin D and albumin, elevated serum phosphorus, osteoprotegerin, and IL6 levels (Table 1).

Risk of bone fractures

Fifty-five (7%) out of 839 KTR had bone fractures during the follow-up period, which were most frequently localized in the upper and lower limbs/hip. The overall incidence of bone fracture was higher in the higher MIS group compared to the lower MIS group (11% vs 5%, $p = 0.002$) (Table 1). The incidences of 1 and 3 or more fractures were also higher in the highest MIS group compared to the lowest MIS group (9% vs. 3% and 1.4% vs. 0.5% respectively, $p = 0.01$) (Table 1).

The association between MIS (as a continuous variable, each one-point increment) and risk of fracture using unadjusted and adjusted logistic and ordinal logistic regression models are presented in Table 2. Higher MIS was associated with a 23% higher crude risk of overall bone fracture (OR [95% CI] 1.23, [1.12–1.34], $p < 0.001$). Even after multivariable adjustment, higher MIS was still associated with 17% and 19% higher risk of fractures in multivariable adjusted and PS adjusted models. Results were similar when modeling outcomes by severity of fractures in ordinal logistic regression analysis (Table 2). Figure 2 shows a linear positive association of MIS as a continuous variable with risk of bone fractures in unadjusted logistic regression using cubic splines.

In subgroup analyses, the association between MIS and bone fractures showed similar results across most examined subgroups (Figs. 3 and 4). Statistically significant interaction was present for age and iPTH, with stronger associations in adjusted models among older patients and in those with iPTH ≥ 150 pg/ml.

Discussion

To the best of our knowledge, this is a first study evaluating the association between the MIS score and new onset clinically detected bone fractures in prevalent kidney transplant recipients. In this prospective observational cohort study, we found that patients with higher MIS had higher incidence of bone fractures. Each one-point increment in MIS score was associated with 23% higher risk of bone fractures. This association remained significant after multivariable adjustments and was stronger in those of older age and higher PTH values.

Here, we reported 7% of our prevalent kidney transplant recipients had a new clinical fracture event during the 2-year follow-up period. A systematic review and meta-analysis, including large number of studies with 262,678 kidney transplant recipients, demonstrated a wide range of 5-year cumulative incidence for fracture from 0.85 to 27% [23]. However, the most recent studies reported an incidence of post-kidney transplant fractures of around 3 to 6%, which is comparable

Table 1 Description of baseline characteristics and outcome data

Parameters	All ($n = 839$)	MIS < 5 ($n = 631$)	MIS ≥ 5 ($n = 208$)	p value
MIS	3 (2–4)	2 (1–3)	6 (5–7)	< 0.001
Demographics				
Age (year)	51 ± 13	50 ± 13	53 ± 12	< 0.001
Gender (male), *n* (%)	472 (56)	382 (60)	90 (43)	< 0.001
Comorbidities				
Hypertension, *n* (%)	780 (93)	587 (93)	193 (93)	0.907
Coronary heart disease, *n* (%)	69 (8)	44 (7)	25 (12)	0.022
Diabetes, *n* (%)	172 (21)	113 (18)	59 (28)	0.001
Smokers, *n* (%)	156 (19)	113 (18)	43 (21)	0.374
Previous history of bone fractures (anytime), *n* (%)	126 (15)	76 (12)	50 (24)	< 0.001
Charlson Comorbidity Index	2 (2–3)	2 (2–3)	3 (2–4)	< 0.001
eGFR (ml/min/1.73 m^2)	55 ± 21	57 ± 20	51 ± 22	< 0.001
Bone–mineral markers				
Phosphorus (mmol/l)	1.05 ± 0.3	1.03 ± 0.3	1.11 ± 0.3	< 0.001
Serum total calcium (mmol/l)	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	0.165
iPTH (pg/ml)	66 (46–99)	65 (46–95)	72 (48–118)	0.063
Alkaline phosphatase (UI/l)	81 (64–103)	80 (64–100)	83 (64–110)	0.141
Osteoprotegerin (pmol/l)	3.63 (2.9–4.6)	3.5 (2.8–4.4)	4.2 (3.2–5.3)	< 0.001
25-OH vitamin D (ng/ml)	10.1 (6.0–14.9)	10.8 (6.5–15.7)	8.4 (4.95–12.2)	< 0.001
FGF-23 (RU/ml)	27.4 (19.8–40.1)	26.4 (19.3–38.5)	29.6 (21.1–48.7)	0.003
Osteocalcin (ng/ml)	35.2 (22.2–52.7)	35.3 (22.2–51.8)	34.2 (21.6–56.5)	0.944
Inflammatory markers				
hsCRP (mg/l)	3 (1.44–6.7)	2.9 (1.5–6.1)	3.5 (1.4–7.9)	0.110
IL6 (pg/ml)	2.0 (1.2–3.5)	1.9 (1.2–3.1)	2.5 (1.5–4.6)	< 0.001
TNF-alpha (pg/ml)	2.0 (1.5–2.8)	2.0 (1.5–2.7)	2.1 (1.5–3.2)	0.061
Nutritional markers				
Serum albumin (g/l)	40.1 ± 3.8	41.4 ± 3.4	38.5 ± 4.0	< 0.001
BMI (kg/m^2)	27.1 ± 4.7	27.5 ± 4.4	25.7 ± 5.4	< 0.001
Abdominal circumference (cm)	98.9 ± 14.4	100.0 ± 13.8	95.5 ± 15.5	< 0.001
Transplant-related data				
Time since last transplant (months)	69 (38–112)	65 (33–110)	80 (49–123)	0.002
Previous time on dialysis (months)	20 (9–38)	19 (9–38)	21 (11–43)	0.182
Total ESRD time (months)	106 (65–154)	103 (63–150)	115 (76–167)	0.015
Deceased donor transplant, *n* (%)	791 (96)	591 (95)	200 (98)	0.029
History of acute rejection, *n* (%)	278 (34)	202 (33)	76 (37)	0.194
Donor age (years)	42 ± 14	43 ± 14	41 ± 14	0.026
Cold ischemic time (min)	1241 ± 345	1227 ± 357	1285 ± 299	0.037
Delayed graft function, *n* (%)	214 (26)	153 (25)	60 (30)	0.127
Immune suppression agents				
Steroid use, *n* (%)	669 (80)	495 (78)	174 (84)	0.105
Prednisolone dose (mg)	5 ± 2	5 ± 2	5 ± 2	0.776
Cyclosporine use, *n* (%)	407 (49)	318 (50)	89 (43)	0.057
Tacrolimus use, *n* (%)	370 (44)	278 (44)	92 (44)	0.965
Azathioprine use, *n* (%)	29 (4)	23 (4)	6 (3)	0.603
MMF use, *n* (%)	674 (80)	530 (84)	144 (69)	< 0.001
Sirolimus use, *n* (%)	61 (7)	34 (5)	27 (13)	< 0.001
Everolimus use, *n* (%)	15 (2)	7 (1)	8 (4)	0.010
MBD medications use				
Vitamin D supplement (ergocalciferol) use, *n* (%)	273 (33)	196 (31)	77 (37)	0.112

Table 1 (continued)

Parameters	All ($n = 839$)	MIS < 5 ($n = 631$)	MIS ≥ 5 ($n = 208$)	p value
Phosphate binders use, *n* (%)	36 (4)	24 (4)	12 (6)	0.225
Bisphosphonate use, *n* (%)	13 (2)	6 (1)	7 (3)	0.014
Thyroid gland medication use, *n* (%)	14 (2)	11 (2)	3 (1)	0.769
Outcomes				
Fracture, *n* (%)	55 (7)	32 (5)	23 (11)	0.002
Number of fractures:				0.010
Once, *n* (%)	40 (5)	22 (3)	18 (9)	
Twice, *n* (%)	9 (1)	7 (1)	2 (1)	
Three or more, (%)	6 (0.7)	3 (0.5)	3 (1.4)	
Fracture localizations:				
Upper limb, *n* (%)	23 (3)	14 (2)	9 (4)	0.106
Lower limb/hip, *n* (%)	23 (3)	13 (2)	10 (5)	0.035
Spine, *n* (%)	1 (0.1)	0 (0)	1 (0.5)	0.081
Rib, *n* (%)	6 (0.7)	4 (0.6)	2 (1)	0.627
Pelvis, *n* (%)	2 (0.2)	1 (0.2)	1 (0.5)	0.014

MIS malnutrition inflammation score, *eGFR* estimated glomerular filtration rate, *iPTH* intact parathyroid hormone, *FGF-23* fibroblast growth factor 23, *hsCRP* high sensitivity C-reactive protein, *IL6* interleukin 6, *TNF-alpha* tumor necrosis factor alpha, *BMI* body mass index, *ESRD* end-stage renal disease, *MMF* mycophenolate mofetil

with our results [4, 24–27]. According to the study by Naylor et al. [4], fractures are most frequently localized in the lower extremity (32.5% lower leg (including tibia, fibula, patella and ankle) and 10.6% hip fractures) and upper extremity (26.9% humerus and 7.5% forearm fractures), which is also in accordance with our data [4]. Older age, female gender, and fall with hospitalization among pre-transplant are classic risk factors, and mineral–bone disorders (MBD) with its laboratory abnormalities (low serum calcium and vitamin D levels; high serum phosphorus, iPTH, bone-specific alkaline phosphatase, and FGF-23 levels) as well as decline in eGFR represent renal-specific risk factors of bone fractures in KTR [27, 28]. In addition, transplant-specific risk factors such as higher cumulative steroid dose, deceased donor transplants, higher HLA-mismatch and persistent MBD after transplantation are the most important cause of bone fractures [24–29]. However, the assessment of nutritional markers (except BMI) as a potential risk factor of bone fractures in kidney transplant population remained insufficiently defined until present time.

Similar to our main results, previous studies showed association between presence of malnutrition–inflammation and higher risk of fractures in nonkidney transplant population. A strong relationship between malnutrition–inflammation and sarcopenia has been found in elderly patients with hip fractures [30]. Hypoalbuminemia was independently associated with 4- to 12-fold higher risk of osteoporosis at different anatomical sites from the unselected nationwide cohort of National Health and Nutrition Examination Survey (NHANES) [31]. In addition, BMI less than 18 kg/m^2 was associated with 40% higher risk of fractures compared to

Table 2 Association between MIS (as a continuous variable, each one-point increment) and risk of fracture using unadjusted and adjusted logistic and ordinal logistic* regression models

Models	Fractures*		Number of fractures**	
	Odds ratio (95%CI)	p value	Odds ratio (95%CI)	p value
Unadjusted ($n = 839$)	1.23 (1.12–1.34)	< 0.001	1.21 (1.12–1.32)	< 0.001
Adjusted model[#] ($n = 825$)	1.17 (1.06–1.29)	0.002	1.16 (1.05–1.27)	0.003
PS adjusted model[$] ($n = 799$)	1.19 (1.07–1.32)	0.001	1.18 (1.06–1.30)	0.002

MIS malnutrition inflammation score, *eGFR* estimated glomerular filtration rate, *iPTH* intact parathyroid hormone, *hsCRP* high sensitivity C-reactive protein, *IL6* interleukin 6, *TNF-alpha* tumor necrosis factor alpha, *MMF* mycophenolate mofetil, *PS* propensity score

*Adjusted logistic regression model

**Ordinal logistic regression model

[#] Adjusted model: Adjusted for age, gender, eGFR, smoking habits, history of pre-transplant bone fractures, and history of acute rejection

[$] PS adjusted model: adjusted for propensity-score (based on logistic regression presented in Supplement Table 1)

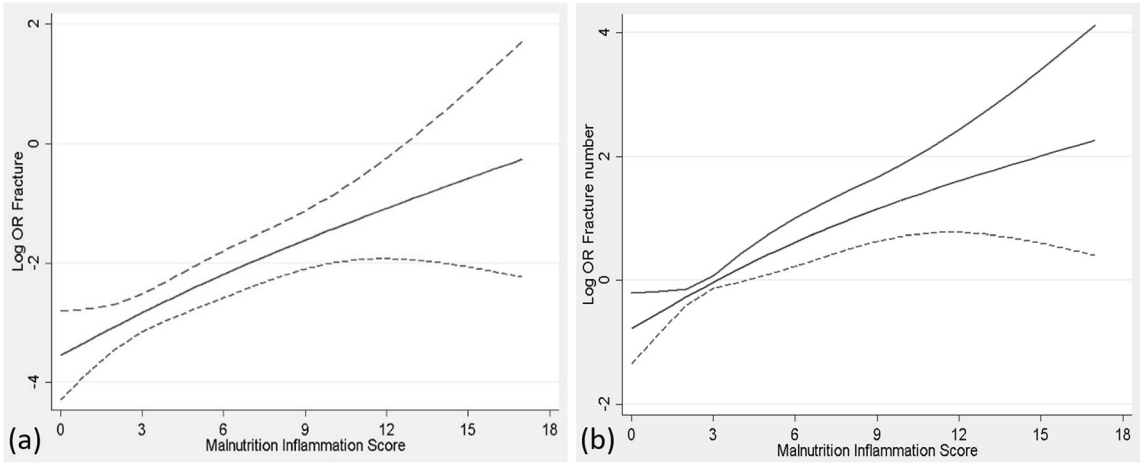

Fig. 2 An unadjusted association of MIS with bone fractures (yes/no) (**a**) and number of fractures (**b**) using cubic splines

BMI 18–25 kg/m^2 in KTR [23]. Although BMI and serum albumin are fair surrogates for protein energy wasting (PEW), MIS consists of elements of SGA, reports on appetite, and also includes liver produced inflammatory proteins (transferrin), which reflect better the complex nutritional and inflammatory status in kidney transplant recipients than any of these items alone [11]. We demonstrated that the presence and severity of PEW are independent risk factors of bone fractures in KTR even after adjustment for known confounders such as female gender, previous history of fracture, smoking, age, and graft function.

In our study, age and iPTH level were important effect modifiers in the association between PEW and risk of fracture. Age-related osteoporosis and fragility fractures are traditional risk factors [32]. Several studies in KTR reported that older age (≥ 45 years) was independently associated with higher risk of bone fractures [3, 25–27, 33], which was also confirmed in our subgroup analysis. In addition, persistent secondary hyperparathyroidism with vitamin D deficiency was found to be risk factors of vertebral fractures after kidney transplantation [34, 35].

Although previous studies in KTR did not examine the frequency of repeated fractures, in our study we observed a 2% incidence of two and more repeated fractures during the 2-year follow-up. These repeated fractures are mostly associated with severe osteoporosis which contributes to further fragility fractures [36]. We found a linear positive association between the severity of PEW and the number of fractures, with each one-point increment in MIS being associated with 21% higher risk of repeated fractures.

There are several potential pathophysiological explanations of the observed associations between PEW and the risk

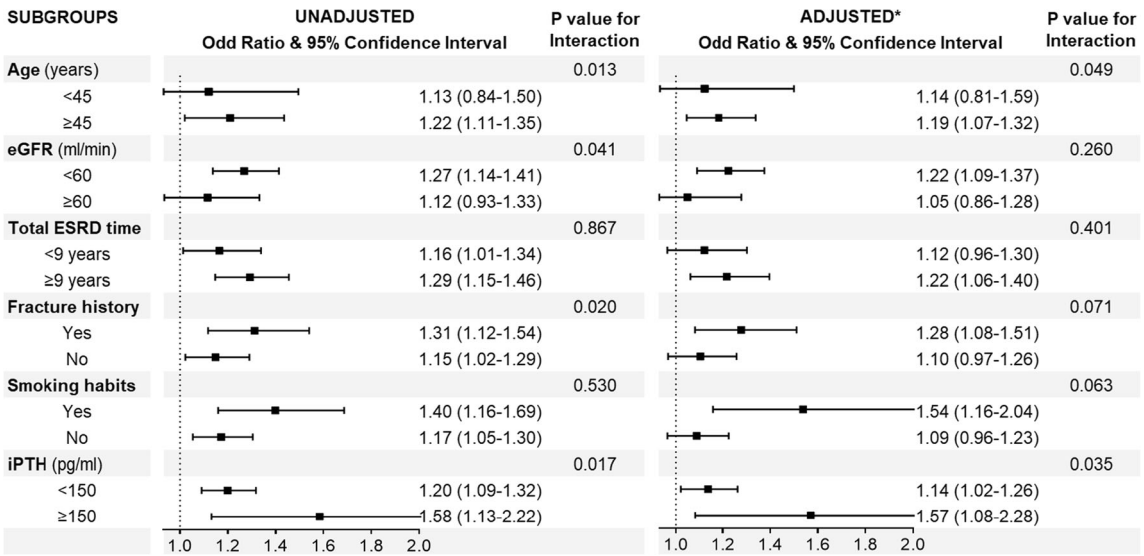

Fig. 3 The association between MIS and risk of fractures in different subgroups using logistic regression models (unadjusted and adjusted model). Adjusted for age, gender, eGFR, smoking habits, history of pre-transplant bone fractures and history of acute rejection (asterisk). MIS malnutrition inflammation score, eGFR estimated glomerular filtration rate, iPTH intact parathyroid hormone, ESRD end-stage renal disease

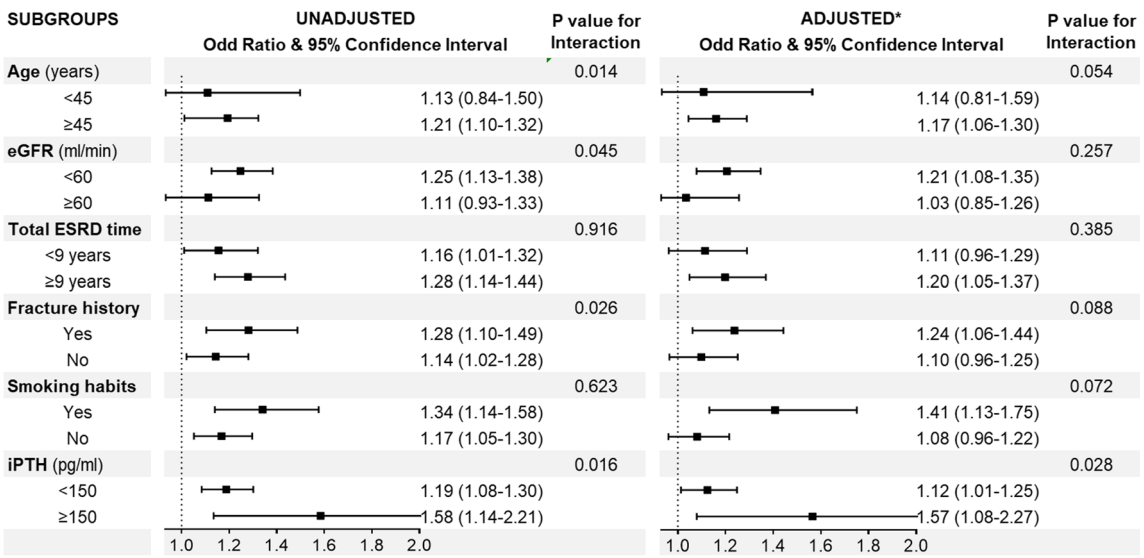

Fig. 4 The association between MIS and number of fractures in different subgroups using ordinal logistic regression models (unadjusted and adjusted model). Adjusted for age, gender, eGFR, smoking habits, history of pre-transplant bone fractures, and history of acute rejection (asterisk). MIS malnutrition inflammation score, eGFR estimated glomerular filtration rate, iPTH intact parathyroid hormone, ESRD end-stage renal disease

of bone fractures. In healthy subjects, bone metabolism (bone-formation and bone-resorption) is closely regulated by the balanced action between osteoblasts and osteoclasts [37]. In inflamed subjects, inflammatory cytokines may destroy this balance and lead to excess re-resorption in favor of bone-formation [15]. The most important regulators of bone remodeling are osteoprotegerin (OPG) and its ligand also known as receptor activator of nuclear factor-kB ligand (RANKL), which is expressed on osteoblasts [38]. Its expression is very sensitive and may be increased by proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukins (IL-1b, IL-6, IL-11, and IL-17) and some other pro-resorptive signals such as glucocorticoids, estrogen deficiency, and PTH excess [15, 16]. Overexpression of the OPG/RANKL system induced by proinflammatory cytokines may result in severe osteoclastogenesis and excessive bone resorption, thereby leading to osteoporosis, decline in bone density and inability to replace the lost bone with new matrix, which increases the risk of fractures [15, 16]. In our cohort, serum osteoprotegerin level and levels of pro-inflammatory markers were higher in patients with high MIS, which support this theory. However, inflammation is unlikely to be the only explanation of the direct association between MIS and fractures, as nutritional components of the PEW could also have major contributions on the observed association. Bone formation is very sensitive to malnutrition, which is associated with a hypercatabolic condition that leads to loss of lean mass and increase the risk of fracture [39]. On the other hand, hypoalbuminemia may induce bone resorption via decreased deposition of albumin in and increased efflux of albumin from spongiosal components of bone [40]. Additionally, hypoalbuminemia affects the homeostasis of calcium-phosphorus, PTH, vitamin D, and estrogens, therefore reducing osteoblast activity and inducing osteoclast activity [41, 42].

There are several strengths and weaknesses of our study. The strengths of our study are the large number of participants with minimal missing data and prospective follow-up and the use of alternative statistical approaches to test our hypothesis, adjusting for important confounders and separately analyzing for repeated bone fractures. However, some important limitations of our study should be recognized. First, this was a single-center study with exclusively Caucasian participants and the results of our study may not be applicable to other racial/ethnic groups. Second, we had a short follow-up period and relatively small number of events, which might have limited the power of our study. Third, detailed information about specific causes and anatomical localizations, severity and received treatments, hospital stay, and outcomes related to fractures are not available in our data. Vertebral fractures can particularly be silent, and our results should be interpreted cautiously in this regard as we did not have information on interval height loss, either. Fourth, serum carboxy-terminal telopeptide was not measured in our patients and, therefore, the relative contribution of increased bone absorption to increased fracture risk remains unknown. Fifth, formal measurements of bone–mineral density with dual X-ray absorptiometry were not available and the contribution of osteopenia or osteoporosis to fractures in our cohort remained undefined. Sixth, the information about fracture was collected by self-report and chart review, and no systematic assessment for fractures was done. Finally, we have only 55 events, which restricted our ability to adjust for important confounders in our main model. However, our approach to adjust for propensity score, based on logistic regression to predict high MIS score,

is the best way to handle this problem and take into account more than just few confounders. Moreover, we also performed different sensitivity analyses to support our message.

Conclusion

In this large prospective study of kidney transplant recipients, higher MIS score was independently associated with higher risk of incident clinically detected bone fractures. Further studies are needed to better understand this association and explore the underlying mechanisms.

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Compliance with ethical standards

The study was approved by the Institutional Ethics Committee (49/2006 and 14-03481-XM) of the Semmelweis University and the University of Tennessee, and all patients provided written and verbal informed consent before enrollment. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism." Patients were examined according to good medical and laboratory practice and in keeping with the recommendations set forth by the Declaration of Helsinki Guidelines for Biomedical Research Involving Human Subjects.

Conflicts of interest None.

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