European Journal of Physical and Rehabilitation Medicine 2025 February;61(1):119-29
DOI: 10.23736/S1973-9087.24.08496-X

ORIGINAL ARTICLE

Association between muscle strength and echogenicity using greyscale ultrasound software: a diagnostic accuracy study in kidney transplant candidates

Carolina ACUÑA-PARDO [1, 2], Elena MUÑOZ-REDONDO [1, 2], Lou DELCROS-FORESTIER [1, 2], Yulibeth G. CURBELO [1], Carlos RODRÍGUEZ-HERNÁNDEZ [1], Delky MEZA-VALDERRAMA [2, 3, 4], Dolores SÁNCHEZ-RODRÍGUEZ [2, 5, 6], Julio PASCUAL [7, 8], Maria J. PÉREZ-SÁEZ [7, 9], Ester MARCO [1, 2, 10] *

[1]Department of Physical Medicine and Rehabilitation, Hospital del Mar, Barcelona, Spain; [2]Rehabilitation Research Group, Hospital del Mar Research Institute, Barcelona, Spain; [3]National Institute of Physical Medicine and Rehabilitation (INMFRE), Panama City, Panama; [4]Department of Physical Medicine and Rehabilitation, Caja de Seguro Social (CSS), Panama City, Panama; [5]Department of Geriatrics, Brugmann University Hospital, Université Libre de Bruxelles, Brussels, Belgium; [6]WHO Collaborating Center for Public Health Aspects of Musculo-Skeletal Health and Ageing, Division of Public Health, Epidemiology and Health Economics, University of Liège, Liège, Belgium; [7]Nephropathies Research Group, Hospital del Mar Research Institute, Barcelona, Spain; [8]Department of Nephrology, Hospital Universitario 12 de Octubre, Madrid, Spain; [9]Department of Nephrology, Hospital del Mar, Barcelona, Spain; [10]Faculty of Health and Life Sciences, Universitat Pompeu Fabra, Barcelona, Spain

*Corresponding author: Ester Marco, Department of Physical Medicine and Rehabilitation, Hospital del Mar, Sant Josep de la muntanya 12, 08024 Barcelona, Spain. E-mail: emarco@psmar.cat

ABSTRACT

BACKGROUND: Advanced chronic kidney disease disrupts the delicate equilibrium between protein anabolism and catabolism, leading to alterations in muscle quantity, quality, and function. Musculoskeletal ultrasound emerges as a promising assessment tool due to its widespread availability and high reliability.
AIM: To evaluate the efficacy of *rectus femoris* (RF) echogenicity, measured using greyscale software, in identifying diminished muscle quality and strength in candidates for kidney transplant.
DESIGN: *Post-hoc* diagnostic accuracy study.
SETTING: Outpatients in a multimodal prehabilitation program pre kidney transplantation (KT).
POPULATION: Patients on the waiting list for KT.
METHODS: Sensitivity, specificity, likelihood ratios and area under the curve (AUC) for diagnostic efficacy of echogenicity (index test) assessed with the ImageJ software greyscale as a potential marker of quadriceps muscle weakness (reference test) were calculated. Muscle weakness was considered as maximal voluntary isometric contraction of the quadriceps (Q-MVIC) <40% of body weight. Other variables included body composition parameters derived from multifrequency electrical bioimpedance, upper limb muscle strength (handgrip), and RF thickness assessed by ultrasound. Statistical tests: Chi-square, t-Student, Pearson correlation coefficients (r), bivariate and multivariate logistic regression models. Statistical significance level ≤0.05.
RESULTS: Of 112 patients (mean age: 63.6, 76% male), 72 (63.7%) exhibited quadriceps weakness, while 80 (70.8%) had some degree of overhydration (extracellular water/total body water ratio >0.390). The echogenicity cut-off point of highest concordance with muscle weakness was 70, boasting a sensitivity of 83%, specificity of 57%, and AUC of 0.671 (CI 95% 0.570-0.772 [P=0.003]). Echogenicity >70 was associated with a 3.4-fold higher risk of muscle weakness (crude OR = 3.4 [CI95% 1.4 to 8.0]), which persisted after adjusting for age, height, weight and RF thickness.
CONCLUSIONS: The RF echogenicity exhibits fair validity in identifying muscle weakness among candidates for KT. However, it cannot be endorsed as a standalone diagnostic tool in this population.

CLINICAL REHABILITATION IMPACT: Early identification of muscle weakness would advance efforts to mitigate morbidity and mortality through targeted measures.

(*Cite this article as:* Acuña-Pardo C, Muñoz-Redondo E, Delcros-Forestier L, Curbelo YG, Rodríguez-Hernández C, Meza-Valderrama D, *et al.* Association between muscle strength and echogenicity using greyscale ultrasound software: a diagnostic accuracy study in kidney transplant candidates. Eur J Phys Rehabil Med 2025;61:119-29. DOI: 10.23736/S1973-9087.24.08496-X)

KEY WORDS: Muscles; Ultrasonography; Muscle strength; Kidney transplantation.

Advanced chronic kidney disease (CKD) leads to systemic hypercatabolism and disrupts the protein synthesis/consumption ratio, creating an "accelerated aging model."[1] This model is characterized by diminished muscle size, quality, and function,[2] coupled with increased fat and fibrotic tissue accumulation, resulting in reduced exercise capacity, muscle strength, and higher risk of falls. Muscle damage in CKD is attributed to multiple factors, including the severity of kidney disease, dialysis treatment, the chronic low-grade inflammation, accumulation of reactive oxygen species and oxidative stress.[3, 4] Consequently, sarcopenia affects up to 55% of kidney transplantation (KT) candidates,[5] substantially impacting their quality of life and overall survival.[6]

The European Working Group on Sarcopenia in Older People (EGWSOP2) recognizes sarcopenia as a progressive skeletal muscle disorder linked to adverse outcomes like falls, fractures, physical disability, and death.[6] Diagnosis relies on two criteria: low muscle strength and loss of muscle quantity and/or quality. Assessing muscle mass, endorsed by EWGSOP, involves using electrical bioimpedance analysis (BIA); additionally, muscle ultrasound is emphasized for its promising accessibility and reliability.[6] The expert group of the International Society of Physical and Rehabilitation Medicine (ISPRM), with special interest in sarcopenia (ISarcoPRM) has launched an algorithm that includes ultrasound for diagnosing sarcopenia.[4]

The SARCopenia measurement by Ultrasound (SARCUS) group recommends the use of ultrasound parameters for assessing muscle quantity and quality.[7, 8] Among the six proposed muscle quality indicators (muscle fascicle length, pennation angle, echogenicity, muscle stiffness, contraction potential, and microcirculation), echogenicity using greyscale softwares has been suggested as a quick and accessible method for estimating muscle quality.[9, 10] Increased muscle echogenicity is associated with a decrease in muscle fiber size and an increase in connective tissue, which can lead to decreased muscle strength. Nevertheless, there is limited research exploring the association between echogenicity and muscle function.

Under the hypothesis that hyper-echogenicity could serve as a marker for muscle dysfunction, this study aimed to determine the degree of agreement between echogenicity of the *rectus femoris* (RF) muscle, assessed with the greyscale of the ImageJ software, and muscle weakness evaluated with maximal voluntary isometric contraction of the quadriceps (Q-MVIC) in KT candidates. The associations of echogenicity with increased risk of muscle weakness were also examined.

Materials and methods

Study design

Post-hoc diagnostic accuracy study using baseline data from the Frailmar study, a clinical trial aimed to assess effectiveness of a multimodal prehabilitation program in kidney transplant (KT) candidates.[11] The study adhered to the recommendations outlined in the Standards for Reporting of Diagnostic Accuracy Studies (STARD).[12]

Study setting

The study was conducted at the Physical Medicine and Rehabilitation Department of a university hospital in Barcelona (Catalonia, Spain).

Participants

The Frailmar cohort included adults on the waitlist for KT from January 2020 to June 2024 referred to an exercise-based prehabilitation program; individuals unable to perform the exercise regimen or with previous muscle diseases were excluded. For the purpose of this accuracy study, having access to baseline BIA and ultrasound information were additional inclusion criteria.

Testing methods

The index test was the measurement of echogenicity of the dominant RF in the lower limb, using the greyscale of the ImageJ software.[13] This software interprets pixels and generates a histogram with a mean echogenicity value, expressed in unitless values from 0 to 255. The muscle as-

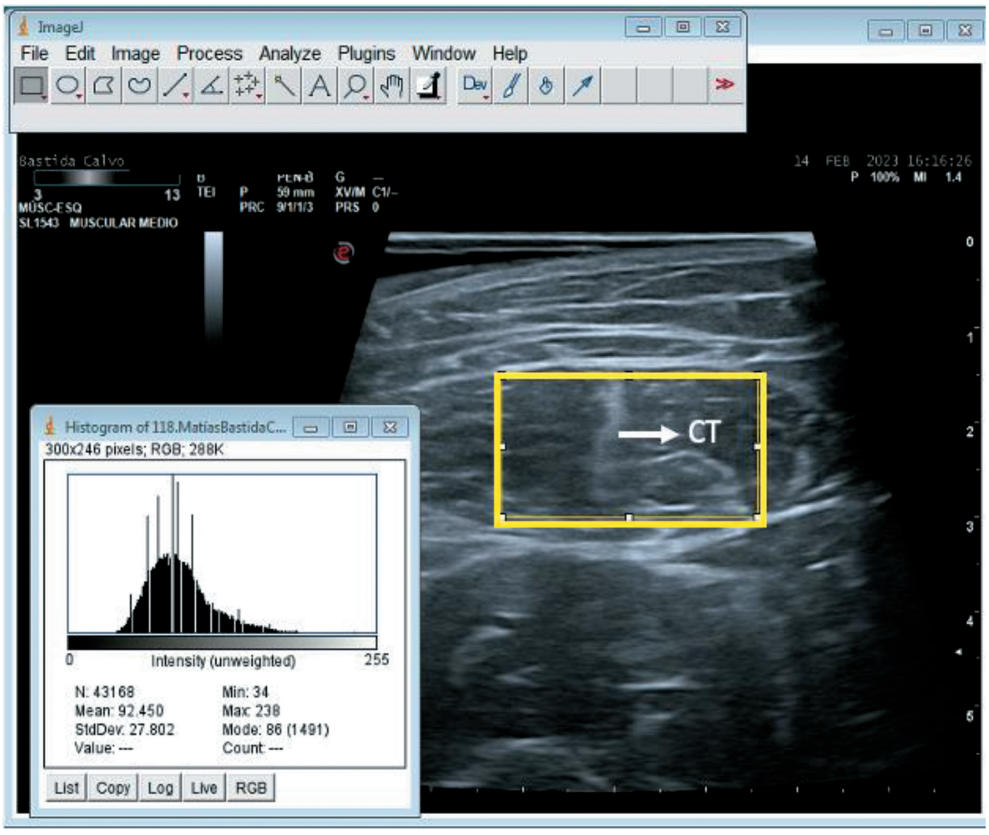

Figure 1.—Screenshot of the Image J Software.
The image depicts the Image J software interface, highlighting a yellow rectangle centered on the *rectus femoris* muscle. The rectangle is strategically positioned to avoid the fasciae, characterized by the presence of the central tendon (CT). Following image analysis, a histogram is generated, displaying the mean echogenicity value obtained from the analysis of image pixels.

sessment was performed with B-mode on MyLab™ Seven (Esaote, Genoa, Italy) by the same experienced researcher. With the patient in a supine position and hips and knees neutrally aligned after a minimum rest of 15 minutes, the linear-array transducer (7.5-12 Hz) was positioned perpendicularly to the lower limb's longitudinal axis at the midpoint between the greater trochanter and the proximal border of the patella.[7] Applying minimal pressure, three images were captured, and the best one was chosen. Two independent evaluators identified a rectangular area for analysis, ensuring the central tendon was at the image's center and excluding muscle fascias (Figure 1).

The reference test was maximal voluntary isometric contraction of the quadriceps (Q-MVIC) in the dominant lower limb. A high-resolution Mecmesin® dynamometer (Slinfold, UK) was employed following a standardized procedure where the patient was seated on a backless bed with hands resting on the knees, had the dominant leg flexed at 90°.[14] The highest value from three reproducible maneuvers was selected and normalized by weight. Values below 40% of body weight indicated low muscle strength.[14]

Other study variables

• Handgrip strength: upper arm muscle strength was evaluated by assessing maximal isometric contraction of the hand's flexor muscles using a digital dynamometer (Jamar Plus®, Nottinghamshire, UK) with standardized methodologies.[14] The patient, seated in a chair without armrests, maintained a 90° flexion at the elbow, slightly away from the body. The analysis considered the highest from three consistent maneuvers (with <10% variability between measurements). Handgrip strength was reported in both absolute numbers and percentages of the reference population.[15]

• Body composition parameters, assessed with the multifrequency BIA device InBody S10® (Biospace, Cerritos, CA, USA), included fat-free mass (kg), musculoskeletal mass (kg), body fat (kg and as a percentage of body weight), total body water (TBW), extracellular water (ECW), intracellular water (ICW), all in liters (L), and the ECW/TBW ratio. Overhydration and dehydration were indicated by ECW/TBW ratio values >0.390 and <0.360, respectively.[16] Decreased fat-free mass was defined as val-

ues <80% of the reference population, and a phase angle below 5º was considered decreased.[17, 18]

• RF muscle thickness (mm) was determined from selected images for the echogenicity study. The mean of three measurements of the distance between the upper and lower aponeurosis in a cross-sectional view was calculated. The normal range was 20-21 mm for men and 16.2 mm for women.[19]

• Exercise capacity was assessed using the 6-minute walk test (6MWT), considered decreased if it was <80% of the predicted value.[20]

• Dialysis modality (hemodialysis, peritoneal dialysis, and non-dialysis) and frailty status assessed using Fried's criteria (involuntary weight loss in the previous year, weakness, exhaustion, slow gait, and low physical activity),[21] were considered as potential confounding factors. Due to the *post-hoc* nature of this diagnostic accuracy analysis, the investigator and evaluators were blinded to the measurements of the reference and index tests. Data on the etiology of CKD, as well as performance in basic and instrumental activities of daily living, were included for descriptive purposes.

Study procedures

The baseline assessment of the Frailmar study involved the collection of demographic, anthropometric, clinical and functional variables. A trained researcher administered all functional tests in the Exercise Prescription Laboratory of the Physical Medicine and Rehabilitation department at a tertiary university hospital. Patients not undergoing dialysis were assessed based on their availability. For those undergoing renal replacement therapy, assessments occurred within 24 hours post-hemodialysis. In the case of peritoneal dialysis, patients were instructed to arrive with an empty peritoneal cavity.

Ethical aspects

The study followed national and international ethical guidelines for human subjects' research, including the Good Clinical Practice guidelines, the Code of Ethics, and the Helsinki Declaration. Personal data handling complied with current legislation in Spain and the General Data Protection Regulation of the Organic Law 3/2018 on data protection. Ethical approval was obtained from the institutional Drug Research Ethics Committee (CEIm) (ref. 2019/8623/I). Before participating, individuals received written information about the study and procedures, providing informed consent by signing relevant documentation.

Statistical analysis

Categorical variables were expressed as absolute values and percentages, while quantitative variables were reported as mean and standard deviation (SD). Normality assumption for quantitative variables was assessed through normal probability plots and the Kolmogorov-Smirnov test, corrected using the Lilliefors test. Diagnostic accuracy properties, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy rate (proportion of true results for index and reference tests), and positive and negative likelihood ratios (LR+ and LR-), were calculated. Validity thresholds considered were: sensitivity or specificity <50% (low validity); sensitivity or specificity <80%, but both values >50% (fair validity); and sensitivity and specificity >80% (good validity).[22] An LR >1 indicates an association between the test result and muscle weakness, while values <1 indicate normal muscle strength; LR+ >10 and LR- <0.1 indicate a relevant change in pretest probability. The intra-rater reliability of the greyscale evaluation was assessed with the intraclass correlation coefficient (ICC).[23]

Contingency tables examined patient distribution based on echogenicity and muscle strength. The area under the curve (AUC) assessed the accuracy of echogenicity values in predicting muscle weakness,[24] with values closer to 1 indicating higher diagnostic accuracy.[25] The optimal threshold was determined using the maximum Youden index (ranging from 0 to 1), where a value of 0 renders the evaluation method useless, while 1 indicates perfect sensitivity and specificity.

Associations between echogenicity and strength/body composition parameters were examined using Pearson correlation coefficients (r). Univariate and multivariate logistic regression analyses were conducted to identify covariates associated with low echogenicity. Crude and adjusted odds ratios (OR) with their 95% confidence intervals (95%CI) were reported. Variables significant in univariate analysis and those clinically relevant were included in the multivariate analysis model. Statistical significance was set at $P \leq 0.05$. All analyses were performed using IBM Statistical Package for Social Sciences (SPSS version 25.0; SPSS, Inc., Chicago, IL, USA) software.

Results

Figure 2 summarizes the study participant flow. One hundred twelve patients (mean age 63.7 years, 76.8% men) were included. Demographic, clinical characteristics, and

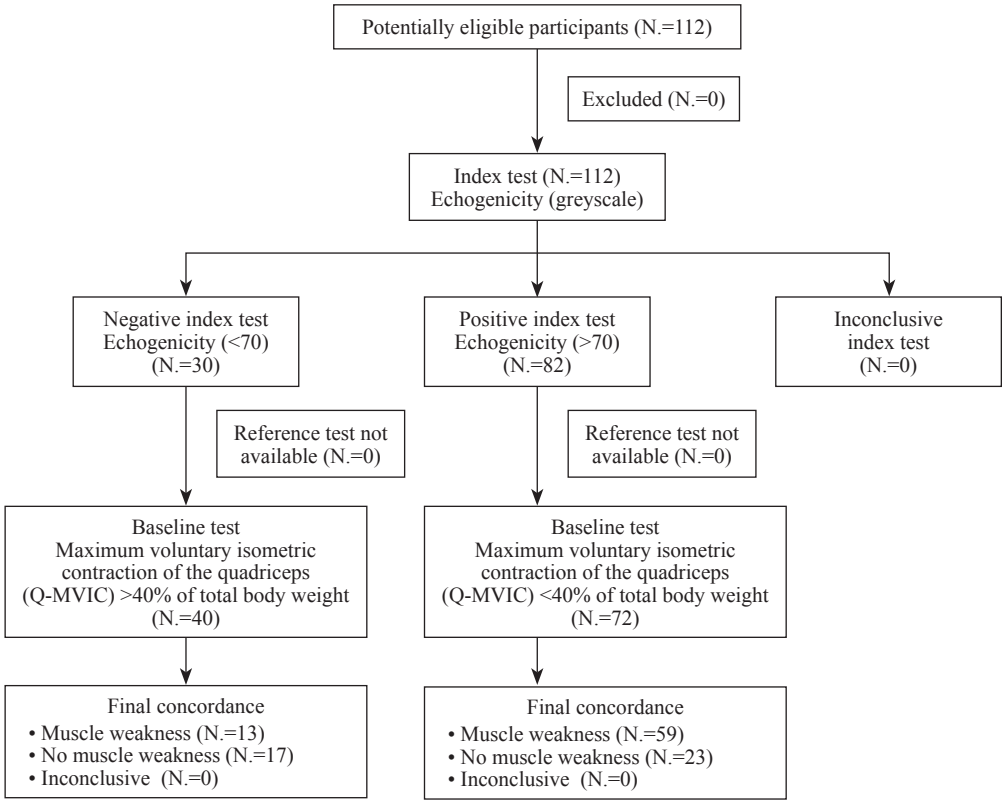

Figure 2.—STARD flow diagram of participants (N.=112).

parameters related to muscle size, quality, function, and body composition are presented in Table I. The mean body mass index was 28.1 (SD 5.1) kg/m^2, classifying as overweight; 16 (14.3%) patients were identified as frail by the Fried phenotype. The most common etiologies of the CKD were idiopathic (32.1%) and metabolic (20.5%). The prevalence of sarcopenia was of 18.9%. Six (5.4%) patients exhibited difficulties for performing activities of daily living (Barthel index <90), and 28 (25%) for performing instrumental activities (Lawton-Brody index <8). Muscle weakness was more prevalent in lower than upper limbs (64.3% *vs.* 50%). The ECW/TBW ratio indicated overhydration, with only 31 patients (27.7%) having normal hydration.

No adverse effects related to the performance of the index and reference tests were observed. The mean echogenicity measured with ImageJ software was 86.2 (SD 24.8). Intrarater reliability was excellent, with an ICC of 0.989. The cut-off point of highest diagnostic accuracy was 70, with a sensitivity of 83%, specificity of 57% (fair validity) and an area under the curve ROC was 0.671 (CI95% 0.570 to 0.772 [P=0.003]), which indicates 67.1% probability that a person with muscle weakness has an echogenicity value >70 under normal hydration conditions (Figure 3A). The same cut-off point of accuracy was obtained for the group of patients with overhydration (N.=81) (Figure 3B). A contingency table was used to show the frequency distribution in patients with muscle weakness according to the echogenicity cut-off point for the overall sample and for the overhydration subgroup (Table II).

The main diagnostic properties of the index test are outlined in Table III. Sensitivity and specificity for detecting muscle weakness were 59.7% and 57.5%, respectively, indicating fair validity. Fagan's nomogram was used to examine pre-and post-test probabilities, indicating a post-test probability of 72% (CI95% 63-79%) for muscle weakness if the index test is positive (echogenicity >70) and 56% (CI95% 46-65%) if negative (normal echogenicity) for the total sample (N.=112) (Figure 4A). Patients with overhydration (N.=81) exhibited a positive post-test probability of 77% (CI95% 71-82%) and 50% (CI95% 30-70%) if the test was negative (Figure 4B).

Table IV presents Pearson correlation coefficients (r) between echogenicity and age, BIA-derived parameters of body composition, and muscle size and function, and quality. Low to moderate correlation was observed for musculoskeletal mass (r=-0.265; P=0.005), fat mass per-

TABLE I.—*Demographic and clinical characteristics of the participants.*

	Sample (N.=112)	Normal range
Age (years)	63.7 (SD 10.7)	-
Sex, meN. (%)	86 (76.8%)	-
Etiology of chronic kidney disease		
Idiopathic	36 (32.1%)	
Metabolic	23 (20.5%)	
Vascular	19 (17%)	
Glomerular	18 (16.1%)	
Hereditary and congenital	9 (8%)	
Tubulointerstitial	5 (4.5%)	
Obstructive uropathies	1 (0.9%)	
Infectious causes	1 (0.9%)	
Dialysis modality, N. (%)		
Hemodialysis	66 (58.9%)	
Peritoneal dialysis	17 (15.2%)	
No renal replacement therapy	29 (25.9%)	-
Body mass index (kg/m^2)	28.1 (SD 5.1)	18.5-25 kg/m^2 [32]
Performance in daily and instrumental activities of daily living		
Barthel Index	97.5 (SD 5.2)	
Barthel Index <90, N. (%)	6 (5.4%)	-
Lawton-Brody Index	7.0 (SD 1.4)	-
Lawton-Brody Index, N. (%)	28 (25%)	
Frailty, Fried's phenotype 3-5 (%)	16 (14.3%)	-
Sarcopenia according the EWGSOP2 criteria, N. (%)	20 (17.9%)	-
Muscle weakness, N. (%)		
Upper limb (*Handgrip* <80% reference population)	56 (50.0%)	-
Lower limb (Q-MVIC <40% body weight)	72 (64.3%)	-
Muscle strength of the dominant side		
Handgrip (kg) [a]	28.2 (SD 10.1)	-
Handgrip (% ref.)	80.7 (SD 23.6)	80-120%[21]
Q-MVIC (Kg) [b]	28.3 (SD 10.1)	-
Q-MVIC (% of body weight)	36.6 (SD 10.7)	<40% of body weight
BIA-derived body composition parameters		
Musculoskeletal mass (kg) [a]	27.7 (SD 6.0)	-
Fat mass (kg) [a]	26.1 (SD 11.6)	-
Fat mass (% of body weight)	32.9 (SD 10.0)	Men 10-20%; women 18-28% [33]
Total body water (L) [b]	37.7 (SD 7.5)	.
Extracellular water (L) [b]	15.0 (SD 2.9)	-
Intracellular water (L) [b]	22.8 (SD 4.6)	-
Ratio extracellular water / total body water	0.397 (SD 0.011)	0.360-0.390 [17, 18]
Phase angle (°)	5.0 (SD 0.9)	5-7 [8]
Muscle size and quality assessed by ultrasound		
Muscle thickness of the dominant forearm (mm) [b]	15.6 (SD 3.9)	13.3-23.5 mm [22]
Muscle thickness of the dominant RF muscle (mm) [b]	17.8 (SD 4.3)	Men 20-31 mm; women 16-24 mm [34]
Echogenicity (greyscale in ImageJ software)	86.2 (SD 24.8)	-

BIA: bioelectrical impedance analysis; Q-MVIC: maximal voluntary isometric contraction of the quadriceps.
[a]Values dependent on age and sex; [b]values dependent on individual characteristics.

centage (r=0.242; P=0.010), intracellular water (r=-0.265; P=0.005), total body water (r=-0.234; P=0.013), extracellular water/total body water ratio (r=0.436; P<0.001), phase angle (r=-0.447; P<0.001), RF thickness (r=-0.304; P=0.001), Q-MVIC (r=-0.325; P<0.001), and 6MWT (r=-0.213; P=0.020).

The univariate analysis based on muscle weakness of the dominant lower limber (Q-MVIC<40% of body weight) revealed statistically significant differences in upper limb strength, musculoskeletal mass, intracellular water (ICW), ECW/TBW ratio, phase angle, RF muscle thickness, and distance travelled in the 6MWT (Table V). In the crude analysis, patients with echogenicity >70 on the greyscale showed a 3.4-fold increased risk of muscle weakness (OR 3.4; 95% CI 1 to 5.5; [P=0.006]). This association persisted in the multivariate analysis after adjusting for age, sex, anthropometric characteristics and RF thickness, as shown in Table VI.

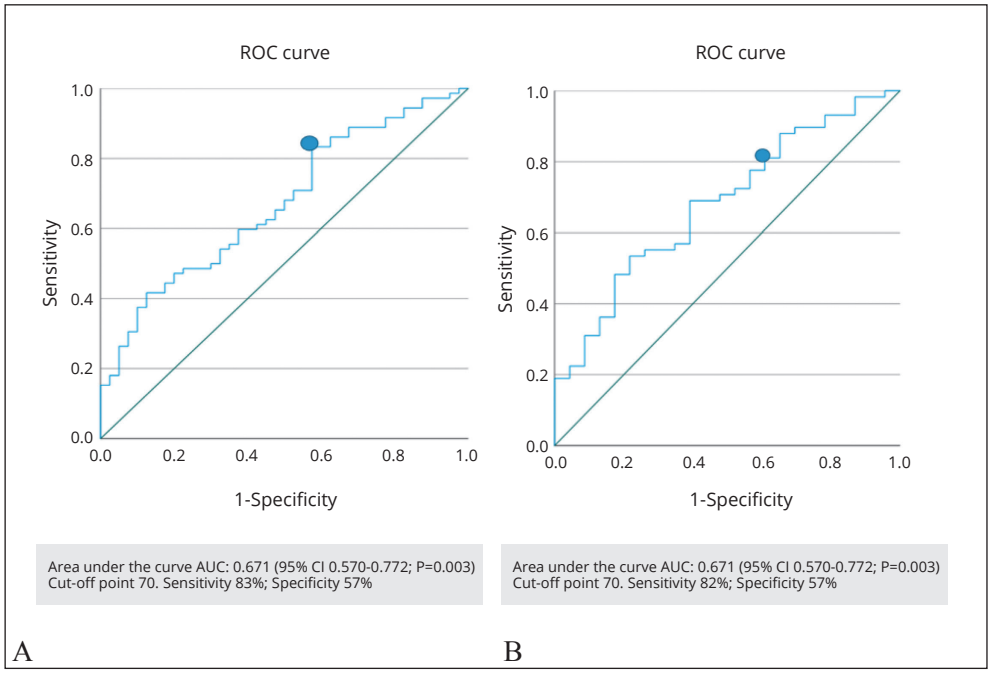

Figure 3.—ROC curve for the detection of lower limb muscle weakness (Q-MVIC, maximal voluntary isometric contraction of the quadriceps) for the total sample (N.=112) (A) and the group of patients with overhydration (N.=81) (B).

TABLE II.—*Contingency table presenting the frequency distribution of echogenicity (cut-off point 70) and muscle weakness of the dominant lower limb assessed by maximal voluntary isometric contraction of the quadriceps in the total sample sample (A), and in the subgroup of patients with overhydration (B).*

A	Reference test: Q-MVIC <40% of total body weight		
Index test: Echogenicity on the greyscale	Muscle weakness (Q-MVIC<40% of weight) (N.=72)	No muscle weakness (Q-MVIC>40% weight) (N.=40)	Total (N.=112)
Hyper echogenicity (>70)	43	17	60
Normal echogenicity (<70)	29	23	52
B	Reference test: Q-MVIC <40% of total body weight		
Echogenicity	Muscle weakness (Q-MVIC <40% of weight) (N.=58)	No muscle weakness (Q-MVIC >40% of weight) (N.=23)	Total (N.=81)
Hyper echogenicity (>70)	50	15	65
Normal echogenicity (<70)	8	8	16

Q-MVIC: maximal voluntary isometric contraction of the quadriceps muscle.

TABLE III.—*Diagnostic properties of the index test (echogenicity >70 on the greyscale using ImageJ software) for detecting muscle weakness in kidney transplant candidates referred to prehabilitation.*

	Echogenicity (Total sample)	Echogenicity B (Patients with overhydration)
Sensitivity	59.7%	86.2%
Specificity	57.5%	34.8%
Positive predictive value	71.7%	76.9%
Negative predictive value	44.2%	50%
Accuracy	58.9%	71.6%
Positive likelihood ratio	1.41	1.32
Negative likelihood ratio	0.70	0.40

Discussion

This diagnostic accuracy study assesses echogenicity of the RF muscle using greyscale with the ImageJ software as a potential marker of muscle weakness in patients with advanced CKD on the waitlist for KT. This analysis is relevant in clinical practice because of its usefulness in screening patients with sarcopenia, whose diagnosis requires the assessment of muscle mass, strength and function.[4]

In an attempt to harmonize the international terminologies, definitions, and diagnostic criteria of sarcopenia, the Global Leadership Initiative on Sarcopenia (GLIS) has re-

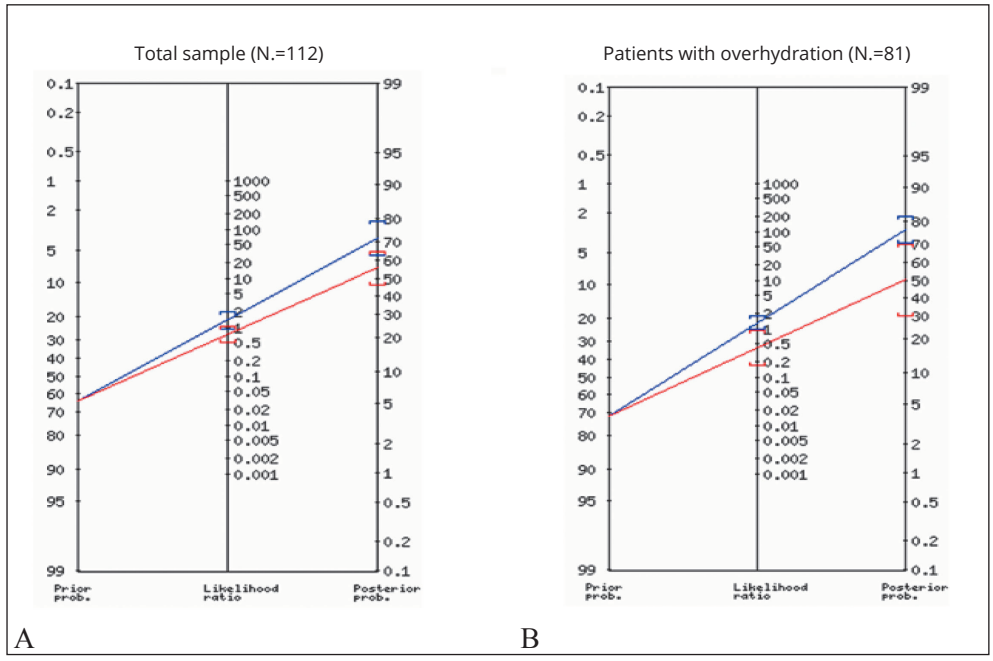

Figure 4.—Positive (blue line) and negative (red line) post-test probability of muscle weakness in the dominant lower limb for the total sample (A), and for the subgroup of patients with overhydration (B). Colors in the online version. Nomograms elaborated with the tool *'Diagnostic Test Calculator'* (available at: araw.mede.uic.edu).

TABLE IV.—*Correlation coefficients between echogenicity (greyscale) and age, parameters of body composition, and muscle size and function.*

	Pearson's coefficient (r)	P
Age	0.102	0.427
Body composition parameters		
Body Mass Index	0.018	0.849
Phase angle (º)	-0.447	<0.001
Fat-free mass (kg)	-0.240	0.011
Musculoskeletal mass (kg)	-0.265	0.005
Fat mass (kg)	0.114	0.230
Fat mass (%)	0.242	0.010
Total body water (L)	-0.234	0.013
Intracellular water (L)	-0.265	0.005
Extracellular water (L)	-0.181	0.057
Extracellular water/total body water ratio	0.436	<0.001
Muscle size and function parameters		
Quadriceps thickness (mm).	-0.304	0.001
Q-MVIC (kg)	-0.297	0.001
Q-MVIC (% body weight)	-0.325	<0.001
Exercise capacity		
6-min-walking test (m)	-0.213	0.020
6-min-walking test (% predicted value)	-0.092	0.341

Q-MVIC: maximal voluntary isometric contraction of the quadriceps.

cently published a conceptual definition that includes reduced muscle mass, strength and muscle-specific strength as the main components of sarcopenia (26). While awaiting the GLIS operational definition of sarcopenia, the EWGSOP2 four-step approach for identifying, diagnosing, and grading severity of sarcopenia remains the most widely accepted (6). The first step of finding potential cases through validated screening instruments or clinical suspicion is crucial, as the effectiveness of the screening tool in a specific population determines whether potentially ill patients will proceed through the pathway and receive adequate care. The GLIS, unlike the EWGSOP2, does not take a position on the necessity of conducting screening for sarcopenia. The expert group of the International Society of Physical and Rehabilitation Medicine (ISPRM), with special interest in sarcopenia (ISarcoPRM) has proposed a screening algorithm for older adults that includes an assessment of muscle function using the Chair Stand Test (CST) ≥12s, and handgrip strength measurement <32 kg (men) and <19 kg (women) (4). If any abnormalities are detected, sarcopenia may be suspected, warranting a sonographic thigh adjustment ratio (STAR) of RF muscle <1.0 (females) and <1.4 (males). If the individual presents with normal STAR values (indicating isolated loss of muscle strength or function), this condition can be classified as "dynapenia." In such cases, it is important to investigate other factors that might affect neuromotor control or depression.[4] The ISarcoPRM algorithm is the first to incorporate ultrasound evaluation in the detection of patients with sarcopenia. Moreover, the ISarcoPRM algorithm highlights the role of the renin-angiotensin-

TABLE V.—*Bivariate analysis according to muscle strength of the dominant lower limb.*

	No weakness (Q-MVIC≥40% of body weight) (N.=40)	Weakness (Q-MVIC≤40% of body weight) (N.=72)	Mean difference (95% CI)	P
Age (years)	61.9 (SD 9.8)	64.6 (SD 11.2)	2.7 (-1.5 to 6.9)	0.198
Body Mass Index (kg/m^2)	27.3 (SD 4.5)	28.6 (SD 5.3)	1.3 (-0.7 to 3.3)	0.207
Upper limb strength:				
Handgrip strength (kg)	34.8 (SD 8.1)	24.5 (SD 9.2)	-10.3 (-13.8 to -6.9)	<0.001
Handgrip strength (% ref.)	89.6 (23.3)	75.8 (SD 22.3)	-13.8 (-22.7 to -5.0)	0.003
BIA-derived body composition parameters				
Musculoskeletal mass	29.3 (SD 5.5)	26.8 (SD 6.2)	-2.5 (-4.9 to -0.2)	0.045
Fat mass (kg)	23.2 (SD 9.7)	27.8 (SD 12.3)	4.6 (0.09 to 9.0)	0.006
Fat mass (% of body weight)	29.5 (SD 8.2)	34.8 (SD 10.5)	5.3 (1.5 to 9.2)	0.065
Total body water (L)	39.5 (SD 6.9)	36.8 (SD 7.8)	-2.7 (-5.6 to 0.2)	0.016
Intracellular water (L)	24.0 (SD 4.2)	22.1 (SD 4.8)	-1.9 (-3.7 to -0.2)	0.033
Extracellular water (L)	15.5 (SD 2.7)	14.7 (SD 3.0)	-0.8 (-1.9 to 0.4)	0.174
Ratio of extracellular water to total body water	0.392 (SD 0.008)	0.400 (SD 0.01)	0.008 (0,004 to 0.012)	<0.001
Phase angle (º)	5.4 (SD 0.7)	4.7 (SD 0.9)	-0.8 (-1.1 to -0.4)	<0.001
Muscle characteristics assessed by ultrasound				
Thickness of the dominant RF (mm)	19.3 (SD 4.1)	16.9 (SD 4.3)	-2.3 (-4.0 to -0.7)	0.006
Echogenicity (range 0-255) unitless	73.4 (SD 24.1)	88.2 (SD 23.5)	14.8 (2.5 to 27.1)	0.010
Exercise capacity:				
6 min walking test (m)	462.1 (SD 104.3)	396.9 (SD 102.8)	-92.2 (-133.0 to -51.4)	<0.001
6 min walk test (% predicted value)	96.2 (SD 18.2)	81.6 (SD 21.4)	-14.6 (-22.6 to -6.6)	<0.001

BIA: bioelectrical impedance analysis; % ref.: percentage of the reference value; Q-MVIC: maximal voluntary isometric contraction of the quadriceps muscles; SD: standard deviation.
Student's *t*-test for independent samples was used to compare quantitative variables, and the Chi-square test was used for categorical variables.

TABLE VI.—*Odds ratios of muscle weakness as a function of echogenicity assessed by a greyscale (dependent variable) adjusted for age, anthropometric and muscle size covariates. Crude and adjusted results.*

	Raw analysis (bivariate)			Multivariate analysis		
Muscle weakness	cOR	95% CI	P	aOR	95% CI	P
Echogenicity (>70)	3.4	1.4 to 8.0	0.006	2.7	1.0 to 7.1	0.041
Age				1.0	0.9 to 1.0	0.745
Sex				8.5	1.3 to 55.3	0.024
Height				1.5	0.0 to 5258	0.918
Weight				1.0	0.9 to 1.6	0.147
RF thickness (mm)				0.9	0.7 to 1.9	0.107

cOR: crude odds ratio; aOR: adjusted odds ratio; RF: *rectus femoris* muscle.

aldosterone system (RAAS), which is over-activated in patients with CKD. This activation has been linked to muscle damage due to the accumulation of reactive oxygen species and oxidative stress, which can induce protein degeneration and muscle atrophy. This process could be counteracted by treatment with angiotensin-converting enzyme inhibitors (ACE inhibitors), which is reflected in improvements in the functional parameters (*e.g.* gait speed or knee extension) included in the algorithm.[4]

Our study population primarily consisted of men due to the specific characteristics of candidates to KT. While the number of female patients in our study is indeed small, we believe it is essential to include their data to provide a comprehensive overview of the population affected by CKD. When comparing baseline characteristics between sexes, setting aside sex-dependent variables such as body composition parameters, muscle strength, RF muscle thickness and meters walked in the 6MWT, significant differences were observed only in the prevalence of frailty (9.3% in men *vs.* 30-8% in women). Further studies are needed to elucidate if these differences on frailty have similar impact on outcomes between different sexes. A previous analysis of the Frailmar cohort revealed a higher risk for women to be frail.[26] Frail women had lower level of education (72.1% *vs.* 57.1%) and economic incomes (20.6% of women had no incomes *vs.* 5.7% of men). Frail men had more comorbidities like chronic obstructive pulmonary disease, ischemic coronary disease, peripheral

and cerebral vasculopathy). Disability for activities of daily living was similar in both sexes, but frail women had more difficulties with instrumental activities of daily living (64.7% in women *versus* 28.6% in men).

In our study, the mean body mass index of 28.1 (SD 5.1) kg/m^2 and body fat percentage of 32.9 (SD 10) are possibly linked to tissue remodeling where healthy muscle transforms into fat and fibrotic tissue, a hallmark of patients with sarcopenia. These changes manifest in muscle echogenicity on the greyscale, exhibiting a negative correlation with Q-MVIC (r=-0.325; P=0.001), the primary focus of this study, and a positive correlation with fat mass percentage (r=0.242; P=0.010). Thus, higher echogenicity corresponds to lower muscle strength and increased fatty infiltration.[4]

The prevalence of lower limb muscle weakness was notably high (64.3%), mirroring findings in a cohort of 8,767 CKD patients with comparable characteristics.[2] This study examined two frailty criteria validated by Fried *et al.*: muscle weakness and gait speed,[21] assessed using Q-MVIC and the 6MWT, respectively. Significant negative correlations were observed for both Q-MVIC (r=-0.325; P<0.001) and 6MWT (r=-0.213; P=0.020).

Echogenicity (>70) was associated with a 3.4-fold increased risk of muscle weakness of dominant lower limb, that persisted after adjusting for age, sex, height, weight, and muscle size. Sex also showed a significant association in the multivariate analysis. These findings suggest that echogenicity is independently associated with muscle weakness, but the effect is not explained solely by age or anthropometric factors. Additionally, the presence of a sex-related effect indicates that gender differences may play a role in the susceptibility to muscle weakness, potentially interacting with echogenicity levels. Further exploration is needed to understand the underlying mechanisms driving these associations.

Limitations of the study

Some study limitations must be taken into account when interpreting the results. First, a potential selection bias may exist, as our study only included KT candidates referred to prehabilitation, preventing the results from being generalized to all patients with advanced CKD. Second, our study lacked data on the duration of renal replacement therapy, a variable impacting patients' physical activity and functionality. Third, the measurement of strength by isometric dynamometry involves volitional maneuvers. Last, but not least important, variations in ultrasound settings and probe positioning may influence muscle echogenicity assessment,[7, 8] which could limit the overall utility of the technique.

Nevertheless, muscle ultrasound is a non-invasive imaging technique readily accessible in rehabilitation settings that can be used to assess muscle mass, quality, and function. Its utility extends beyond diagnostics, proving valuable in monitoring patients undergoing muscle training.[8] Regardless of the existing association between echogenicity and muscle strength, the measurement of echogenicity cannot and should not replace the assessment of strength using standardized systems such as isometric dynamometry. Nevertheless, assessing muscle echogenicity can be useful for a number of reasons. First, it can be used to assess muscle quality independently of muscle size. This is important because muscle quality can decline with age and sarcopenia, even when muscle size is preserved. Second, muscle echogenicity can be used to track changes in muscle quality over time. This can be useful for monitoring the effectiveness of interventions to improve muscle quality, such as exercise or resistance training. Third, muscle echogenicity can be used to identify individuals who are at risk of muscle weakness or sarcopenia. This information can be used to develop targeted interventions to prevent or delay muscle loss.

Conclusions

In this preliminary analysis, using muscle weakness as a reference test, the cut-off point of >70 of the greyscale showed fair validity for identifying patients with muscle weakness. However, pending further studies, it cannot be recommended as a standalone screening tool for muscle weakness. Nonetheless, it may hold significant value as a screening method for sarcopenia.

References

1. Moorthi RN, Avin KG. Clinical relevance of sarcopenia in chronic kidney disease. Curr Opin Nephrol Hypertens 2017;26:219–28.

2. Wilkinson TJ, Miksza J, Yates T, Lightfoot CJ, Baker LA, Watson EL, *et al.* Association of sarcopenia with mortality and end-stage renal disease in those with chronic kidney disease: a UK Biobank study. J Cachexia Sarcopenia Muscle 2021;12:586–98.

3. Sabatino A, Cuppari L, Stenvinkel P, Lindholm B, Avesani CM. Sarcopenia in chronic kidney disease: what have we learned so far? J Nephrol 2021;34:1347–72.

4. Kara M, Kaymak B, Frontera W, Ata AM, Ricci V, Ekiz T, *et al.* Diagnosing sarcopenia: functional perspectives and a new algorithm from the ISarcoPRM. J Rehabil Med 2021;53:jrm00209.

5. Mori K, Nishide K, Okuno S, Shoji T, Emoto M, Tsuda A, *et al.* Impact of diabetes on sarcopenia and mortality in patients undergoing hemodialysis. BMC Nephrol 2019;20:105.

6. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, *et al.*; Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 2019;48:16–31.

7. Perkisas S, Baudry S, Bauer J, Beckwée D, De Cock AM, Hobbelen H, *et al.* Application of ultrasound for muscle assessment in sarcopenia: towards standardized measurements. Eur Geriatr Med 2018;9:739–57.

8. Perkisas S, Bastijns S, Baudry S, Bauer J, Beaudart C, Beckwée D, *et al.* Application of ultrasound for muscle assessment in sarcopenia: 2020 SARCUS update. Eur Geriatr Med 2021;12:45–59.

9. Pillen S, van Dijk JP, Weijers G, Raijmann W, de Korte CL, Zwarts MJ. Quantitative gray-scale analysis in skeletal muscle ultrasound: a comparison study of two ultrasound devices. Muscle Nerve 2009;39:781–6.

10. Zaidman CM, Holland MR, Anderson CC, Pestronk A. Calibrated quantitative ultrasound imaging of skeletal muscle using backscatter analysis. Muscle Nerve 2008;38:893–8.

11. Pérez-Sáez MJ, Morgado-Pérez A, Faura A, Muñoz-Redondo E, Gárriz M, Muns MD, *et al.* The FRAILMar Study Protocol: Frailty in Patients With Advanced Chronic Kidney Disease Awaiting Kidney Transplantation. A Randomized Clinical Trial of Multimodal Prehabilitation. Front Med (Lausanne) 2021;8:675049.

12. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, *et al.*; STARD Group. STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies. Clin Chem 2015;61:1446–52.

13. Schroeder AB, Dobson ET, Rueden CT, Tomancak P, Jug F, Eliceiri KW. The ImageJ ecosystem: open-source software for image visualization, processing, and analysis. Protein Sci 2021;30:234–49.

14. Meza-Valderrama D, Chaler J, Marco E. [Evaluation of muscle strength in rehabilitation: from subjective assessment scales to instrumental examinations]. Rehabilitacion (Madr) 2021;55:2–4. [Spanish].

15. Luna-Heredia E, Martín-Peña G, Ruiz-Galiana J. Handgrip dynamometry in healthy adults. Clin Nutr 2005;24:250–8.

16. Lee Y, Kwon O, Shin CS, Lee SM. Use of bioelectrical impedance analysis for the assessment of nutritional status in critically ill patients. Clin Nutr Res 2015;4:32–40.

17. Di Vincenzo O, Marra M, Di Gregorio A, Pasanisi F, Scalfi L. Bioelectrical impedance analysis (BIA) -derived phase angle in sarcopenia: A systematic review. Clin Nutr 2021;40:3052–61.

18. Marco E, Pérez-Sáez MJ, Muñoz-Redondo E, Curbelo YG, Ramírez-Fuentes C, Meza-Valderrama D, *et al.*; FRAILMar Study Group. Phase angle as surrogate marker of muscle weakness in kidney transplant candidates referred to prehabilitation. Nutrients 2024;16:2245.

19. Miyamoto S, Nagaya N, Satoh T, Kyotani S, Sakamaki F, Fujita M, *et al.* Clinical correlates and prognostic significance of six-minute walk test in patients with primary pulmonary hypertension. Comparison with cardiopulmonary exercise testing. Am J Respir Crit Care Med 2000;161:487–92.

20. Committee AT. on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS Statement: guidelines for the six-minute walk test. Am J Respir Care Med. 2002;166:111–7.

21. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, *et al.*; Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 2001;56:M146–56.

22. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics 1977;33:363–74.

23. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med 2016;15:155–63.

24. Carrero JJ, Johansen KL, Lindholm B, Stenvinkel P, Cuppari L, Avesani CM. Screening for muscle wasting and dysfunction in patients with chronic kidney disease. Kidney Int 2016;90:53–66.

25. Altman DG, Bland JM. Diagnostic tests 3: receiver operating characteristic plots. BMJ 1994;309:188.

26. Pérez-Sáez MJ, Arias-Cabrales CE, Dávalos-Yerovi V, Redondo D, Faura A, Vera M, *et al.*; for the FRAIL-MAR Study Group. Frailty among chronic kidney disease patients on the kidney transplant waiting list: the sex-frailty paradox. Clin Kidney J 2021;15:109–18.

27. Kirk B, Cawthon PM, Arai H, Ávila-Funes JA, Barazzoni R, Bhasin S, *et al.*; Global Leadership Initiative in Sarcopenia (GLIS) group. The Conceptual Definition of Sarcopenia: Delphi Consensus from the Global Leadership Initiative in Sarcopenia (GLIS). Age Ageing 2024;53:052.

Conflicts of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Funding

The FRAILMAR project was supported by FIS-FEDER PI19/00037 (ISCIII). The researcher MP-S has been granted by the Spanish Society of Transplantation.

Authors' contributions

Maria J. Pérez-Sáez and Ester Marco share senior authorship. Carolina Acuña-Pardo wrote the first draft of the manuscript; Elena Muñoz-Redondo, Yulibeth G. Curbelo, and Carlos Rodríguez-Hernández prepared the literature review; Elena Muñoz-Redondo, Lou Delcros-Forestier, and Delky Meza-Valderrama participated in data collection; Carolina Acuña-Pardo, Delky Meza-Valderrama, Dolores Sánchez-Rodríguez and Ester Marco analyzed the data; Dolores Sánchez-Rodríguez, Maria José Pérez-Sáez, Julio Pascual, and Ester Marco revised the manuscript. Elena Muñoz-Redondo made substantial contributions to this research. This manuscript is part of Elena Muñoz-Redondo's PhD project 'Effects of prehabilitation in patients awaiting kidney transplantation' in the PhD program of Medicine, Medicine Department, Universitat Autònoma de Barcelona, Catalonia, Spain. All authors read and approved the final version of the manuscript.

History

Article first published online: December 16, 2024. - Manuscript accepted: November 21, 2024. - Manuscript revised: July 26, 2024. - Manuscript received: March 7, 2024.