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Myosteatosis as an independent risk factor for mortality after kidney allograft transplantation: a retrospective cohort study

Antoine Morel[1,2†], Yaniss Ouamri[1,3†], Florence Canouï-Poitrine[1,4], Sébastien Mulé[1,3], Cécile Maud Champy[1,5], Alexandre Ingels[1,5], Vincent Audard[1,2], Alain Luciani[1,3], Philippe Grimbert[1,2], Marie Matignon[1,2], Frédéric Pigneur[1,3‡] & Thomas Stehlé[1,2*‡]

[1]*Univ Paris Est Créteil, Institut National de la Santé et de la Recherche Médicale (INSERM), Institut Mondor de Recherche Biomédicale (IMRB), Créteil, France;* [2]*Assistance Publique des Hôpitaux de Paris (AP-HP), Hôpitaux Universitaires Henri Mondor, Service de Néphrologie et Transplantation, Fédération Hospitalo-Universitaire "Innovative Therapy for Immune Disorders", Créteil, France;* [3]*Assistance Publique des Hôpitaux de Paris (AP-HP), Hôpitaux Universitaires Henri-Mondor, Service d'Imagerie Médicale, Créteil, France;* [4]*Clinical Epidemiology and Ageing Unit (CEpiA), Institut Mondor de Recherche Biomédicale, Paris-Est University, Créteil, France; and* [5]*Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpitaux Universitaires Henri Mondor, Service d'Urologie, Groupe Hospitalier Henri-Mondor/Albert Chenevier, Créteil, France*

Abstract

Background Patients with end-stage renal disease may display both a loss of skeletal muscle mass and an increase in muscle fat deposits. We aimed to analyse the impact of low skeletal muscle mass index (SMI, surrogate marker of sarcopenia) and low muscle density (MD, surrogate marker of myosteatosis) on patient survival after kidney transplantation (KT).

Methods In a retrospective cohort of 200 kidney transplant recipients (KTr), we measured on an unenhanced cross-sectional computed tomography scan taken at the level of the third lumbar vertebra within the previous year or at the time of KT, both SMI (muscle cross-sectional area normalized for height^2, reported in cm^2/m^2) and MD (mean attenuation of muscle cross-sectional area, expressed in Hounsfield units). We determined age-specific and sex-specific normality thresholds on 130 healthy subjects. The baseline factors associated with low MD were assessed by logistic regression analysis. Cox proportional hazard univariable and multivariable models were constructed to identify predictive factors of patient survival.

Results Among the 200 patients of the cohort, 123 were male (62%), and mean age was 54.8 ± 13.8 years. A total of 181 KTr required renal replacement therapy before KT (91%), and 36 KTr (18%) received repeat kidney transplant after previous failed KT. Mean MD was 30.6 ± 9 HU in men and 29.7 ± 8.3 HU in women, whereas SMI was 49.7 ± 8.6 cm^2/m^2 in men and 42.3 ± 7.3 cm^2/m^2 in women. MD was below the 2.5th percentile for the healthy population in 49 KTr (25%), defining the myosteatosis group, while SMI was below the 2.5th percentile for the reference population in 10 KTr (5%). Independent risk factors for myosteatosis were two or more KT [adjusted odds ratio (aOR) 5.2, 95% confidence interval (95% CI): 2.22–12.4, $P = 0.0001$], a history of stroke (aOR 3.7, 95% CI: 1.30–10.7, $P = 0.015$), and body mass index > 25 kg/m^2 (aOR 2.94, 95% CI: 1.4–6.18, $P = 0.004$). Myosteatosis was independently associated with mortality [adjusted hazard ratio (aHR) 2.12, 95% CI: 1.06–4.24, $P = 0.033$], as were cardiovascular disease (HR 2.06, 95% CI: 1.02–4.15, $P = 0.043$) and age (aHR 1.06, 95% CI: 1.03–1.09, $P = 0.0003$). Low SMI was not associated with mortality.

Conclusions Myosteatosis, which was more prevalent than low skeletal muscle mass, might be an important prognostic marker in patients undergoing KT.

Keywords Myosteatosis; Kidney transplantation; Mortality risk factors; Prognosis; CT scan

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*Correspondence to: Dr Thomas Stehlé, MD, Assistance Publique des Hôpitaux de Paris (AP-HP), Hôpitaux Universitaires Henri Mondor, Service de Néphrologie et Transplantation, Fédération Hospitalo-Universitaire "Innovative Therapy for Immune Disorders", 51 avenue du Maréchal de Lattre de Tassigny, 94000 Créteil, Île-De-France, France. Phone: +33149814451, Email: thomas.stehle@aphp.fr
†These authors equally contributed to this work as first authors.
‡These authors equally contributed to this work as last authors.

Introduction

Kidney transplantation (KT) remains the best treatment for end-stage renal disease (ESRD), resulting in a longer life expectancy than dialysis.[1] Nevertheless, the life expectancy of KT recipients (KTr) remains lower than that in general population, with mean loss of more than 12 years of life.[2] The primary cause of kidney allograft loss is death with a functioning KT,[3] and several clinical scores have been developed for predicting the risk of death in this population. These scores generally consider recipient age, time on dialysis, and the comorbidities of the recipient, including diabetes mellitus and history of cardiovascular events. Some also include biological data (albuminaemia and haemoglobinaemia) and donor characteristics, such as age and the use of extended criteria.[4] However, these scores remain imperfect for predictive purposes and there is a need to identify new risk factors for mortality in the context of KT.

Chronic kidney disease (CKD) is associated with sarcopenia, defined as a loss of skeletal muscle mass and impaired muscle function.[5] This muscle damage has already been associated with harmful consequences at all stages of CKD.[5,6] In KTr, low muscle mass is associated with graft loss and mortality.[7,8] In addition to this loss of skeletal muscle mass, there may be a qualitative impairment of muscle structure in CKD. The infiltration of fat into the skeletal muscle, myosteatosis, is one such structural disorder of muscle.[9] In CKD patients not on dialysis, myosteatosis is associated with impaired physical performance.[10] In patients undergoing peritoneal dialysis, an association between myosteatosis and cardiovascular disease has recently been described.[11] Myosteatosis is now well recognized to be a risk of death in several contexts, including cancer,[12] orthotopic liver transplantation,[13] or mechanical ventilation in intensive care,[14] but no data are available concerning the impact of myosteatosis on mortality in ESRD and KT.

Muscle mass can be quantified by various non-invasive methods, including the use of anthropometric data, the determination of urinary creatinine excretion rate, serum creatinine concentration in dialysis patients, impedancemetry, or dual-energy X-ray absorptiometry.[15] Computed tomography (CT) imaging has emerged as a non-invasive tool for evaluating the quantity and quality of skeletal muscle.[15,16] One of the key advantages of CT scans is that they can provide information about not only the mass of muscle but also its quality. Analysis of abdominal CT sections at the third lumbar vertebra (L3) provides surrogate markers for skeletal muscle mass and myosteatosis.[17] Total muscle mass can be estimated by dividing total lumbar muscle cross-sectional area (CSMA) through the middle of L3 by square size (L3 skeletal muscle mass index: SMI).[17] Mean skeletal muscle density (MD) can be used as a complementary approach for measuring myosteatosis.

Methods

Study design

We conducted a retrospective cohort study to analyse the prognosis value of low SMI and MD on patient survival after KT.

Setting and population

All KTr undergoing transplantation at our centre between January 2014 and December 2017, for whom an abdominal CT scan taken within the previous year or up to 14 days following KT was available, were considered for inclusion. There were no exclusion criteria other than the absence of a CT scan. The institutional review board (*Comité de Protection des Personnes Ile de France IV*) approved this single-centre study (IRB 00003835). The research was conducted in accordance with the Declaration of Helsinki, and 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'.

Study endpoints

The primary endpoint was patient survival after transplantation. Secondary endpoints included the causes of myosteatosis and low SMI, death-censored kidney allograft survival, delayed graft function, estimated glomerular filtration rate (eGFR) within 12 and 24 months after KT, the incidence of acute rejection, community-acquired infections, and opportunistic infections (OI).

Variables

Kidney allograft loss was defined as a need of chronic dialysis. Donors were considered to be extended-criteria donors (ECDs) if they were >60 years old or between 50 and 60 years of age, with two of the three following criteria: hypertension;

pre-retrieval serum creatinine concentration > 1.50 mg/dL; and a cerebrovascular cause of brain death.[18] We calculated eGFR with the CKD-EPI equation.[19] Community-acquired infection was considered in cases of infection requiring hospitalization. OI were defined according to international guidelines.[20]

Muscle density and skeletal muscle mass index measurements at the third lumbar vertebra

All CT examinations were performed on one of the three Multi-Detector CT machines in our radiology department: Lightspeed VCT®, Discovery CT®, and Revolution CT® (GE Healthcare, Milwaukee, WI, USA), according to a standardized protocol. CT imaging parameters were as follows: unenhanced acquisition, 120 kV voltage, filtered back projection (FBP) image reconstruction without iterative reconstruction, 1.25 mm contiguous reconstructed slice thickness, and soft filter kernel. Segmentations were performed with a dedicated post-treatment station (Advantage Window v4.7; GE Healthcare, Buc, France). A pre-established attenuation threshold (−29 to +150) HU was selected,[17] and the CSMA —defined as the total muscle area (including the external and internal obliques, paraspinal, rectus abdominis, transversus abdominis, and psoas muscles) measured on an axial section through the middle of L3—was segmented semi-automatically, as previously reported.[21] CSMA was normalized for height^2 and reported as the SMI in cm^2/m^2.[17] MD was defined on the basis of the attenuation of CSMA at L3 and is expressed in Hounsfield units (HU) (*Figure* 1).[22]

Determination of muscle density and skeletal muscle mass index thresholds

There are no standardized cut-offs for CT-scan-defined myosteatosis,[23] particularly in the context of CKD. We therefore determined age-specific and sex-specific normality thresholds on the basis of data for 130 healthy subjects who underwent functional explorations of the kidney in our department between July 2016 and February 2020. We used linear regression to model MD and SMI according to age, for both sexes. Patients with an MD or SMI below the lower limit

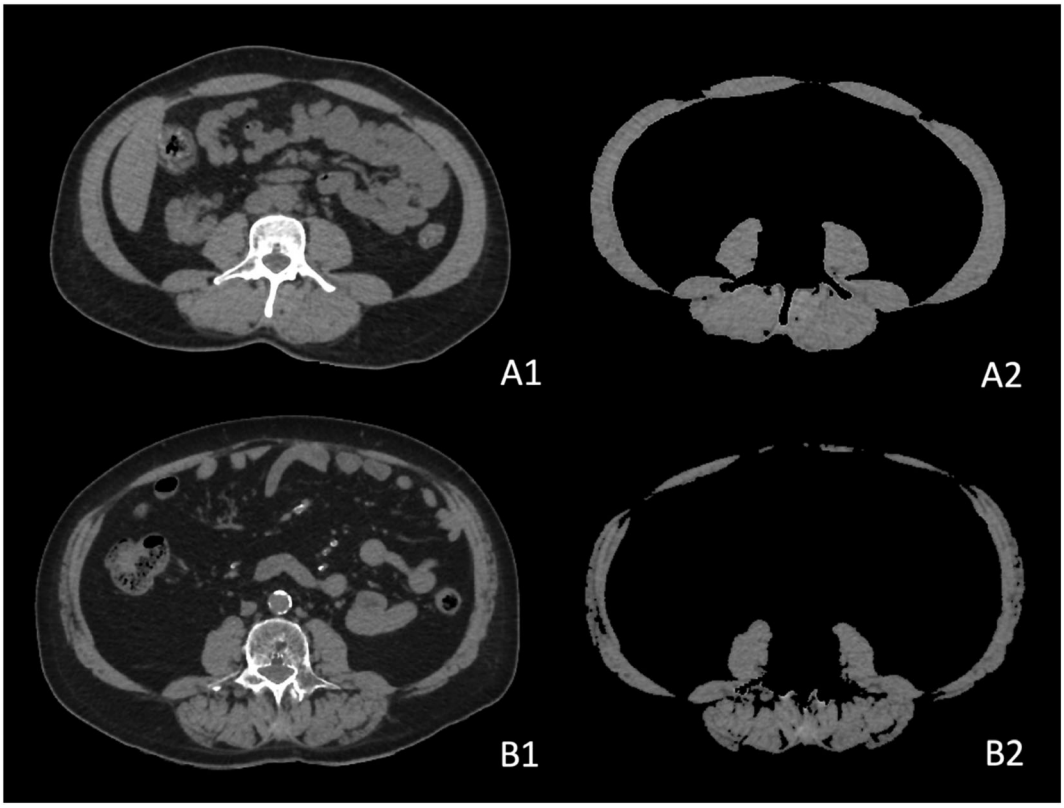

Figure 1 Unenhanced computed tomography scan taken at the level of the middle of the third lumbar vertebra, for two male kidney transplant recipients (A and B) before *(A1 and B1)* and after *(A2 and B2)* the segmentation of total lumbar muscle cross-sectional area. The two patients have similar skeletal muscle index: 50.4 cm^2/m^2 for Patient A and 54.3 cm^2/m^2 for Patient B. In contrast, their muscle densities are different: Patient A has a normal muscle density (43.8 HU), whereas Patient B is considered to have myosteatosis (21.3 HU) according to our criteria.

of the prediction interval for 95% of healthy subjects were considered to have a low MD or low SMI, respectively.

Statistical methods

Categorical data are expressed as percentages, and continuous variables are expressed as medians and interquartile ranges [IQR] or as mean (and standard deviation, SD), as appropriate. We compared both continuous and categorical variables in *t*-tests or Mann–Whitney tests, and χ^2 tests or Fisher's exact tests, as appropriate.

The baseline factors associated with low MD were assessed by logistic regression analysis. The factors identified as significant in the univariate analysis (with a *P*-value threshold of 0.2) were included in a multivariable model, which was then subjected to stepwise backward elimination, with an exclusion threshold of 5%, until the final model was obtained. Results are expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

Survival curves were generated for patients and kidney allografts by the Kaplan–Meier method, with log-rank tests used for comparisons. Cox proportional hazard univariable and multivariable models were constructed to identify factors predictive of patient survival. All potential predictive variables yielding associations with a *P*-value < 0.2 in univariable analysis were included as covariates in the multivariable model, to which a backward conditional selection procedure, with a 5% exclusion threshold, was applied until the final model was obtained. Results are expressed as hazard ratios (HRs) with 95% CI.

Values of $P < 0.05$ in two-tailed tests were considered statistically significant. All analyses were conducted with XL Stat software and Stata v15.0 (StataCorp, College Station, TX, USA).

Results

Patient characteristics

We included 200 of the 409 KTr who underwent transplantation between January 2014 and December 2017. The baseline characteristics of both the KTr and donors are shown in *Table* 1. Most of the recipients were male (*N* = 123; 62%). The recipients had a mean age of 54.8 ± 13.8 years, and the donors had a mean age of 57.6 ± 17. In total, 26 (13%) recipients were obese, and 36 (18%) had already undergone KT at least once before. Almost all the patients were treated with immunosuppressive agents for induction (*N* = 198; 99%), mostly antithymocyte globulin (*N* = 138; 69%). The combinations used for maintenance immunosuppressive therapy included calcineurin inhibitors (*N* = 182; 91%), mycophenolic acid (*N* = 165; 83%), and steroids (100%).

Identification of patients with low muscle density and low skeletal muscle mass index in the kidney transplant recipient groups

The population of 130 healthy subjects analysed to determine the thresholds for low MD and low SMI consisted of 54 men and 76 women. These subjects underwent functional explorations of the kidney in our department: GFR measurement (*N* = 87; 67%), mostly to assess eligibility for kidney donation (*N* = 79), or calcium load tests for suspected primary hyperparathyroidism (*N* = 43; 33%). They had no relevant medical history (*N* = 57; 44%) other than uncomplicated hypertension (*N* = 27; 21%), non-morbid obesity [body mass index (BMI) 30–35] (*N* = 21; 16%), or urolithiasis (*N* = 35; 27%). Their clinical characteristics are provided in Supporting Information, *Table* S1. Mean SMI was 54.8 ± 7.9 in men and 41.7 ± 5.5 in women. Mean MD was 43.8 ± 7.7 in men and 37.0 ± 8.2 in women. In this population, we used in both sexes linear regression to model the association between age and MD (*Figure* 2A) and between age and SMI (*Figure* 2B).

Kidney transplant recipients with MD or SMI below the lower limits of the 95% prediction intervals of the linear regression were considered to have low MD or low SMI, respectively (*Figure* 2A and 2B).

In kidney allograft recipients, MD was 30.6 ± 9 HU in men and 29.7 ± 8.3 HU in women, whereas SMI was 49.7 ± 8.6 cm^2/m^2 in men and 42.3 ± 7.3 cm^2/m^2 in women (*Table* 1). MD was below the 2.5th percentile for the healthy population in 49 KTr (25%) (*Figure* 2A), defining the myosteatosis group. SMI was below the 2.5th percentile for the reference population in 10 KTr (5%) (*Figure* 2B), defining the low skeletal muscle mass group.

Myosteatosis group analysis

Patients with myosteatosis had a higher BMI than patients with a normal MD (26.4 ± 4.5 vs. 24.7 ± 4.3, *P* = 0.024), a longer duration of renal replacement therapy (RRT) (58 [32–77] vs. 47 [22–69], *P* = 0.046), more frequently had a history of stroke (16% vs. 7%, *P* = 0.048), and were more likely to have had at least one previous KT before that considered here (33% vs. 13%, *P* = 0.0046) (*Table* 1). In multivariate analysis, the independent risk factors for myosteatosis were at least one previous KT [adjusted OR (aOR) 5.2, 95% CI: 2.22–12.4, *P* = 0.0001], a history of stroke (aOR 3.7, 95% CI: 1.30–10.7, *P* = 0.015), and BMI $>$ 25 kg/m^2 (aOR 2.94, 95% CI: 1.4–6.18, *P* = 0.004) (*Table* 2).

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Table 1 Patient characteristics according to myosteatosis status, as defined by computed tomography scan

Variables	Whole cohort, *N* = 200	Low muscle density, *N* = 49	Normal muscle density, *N* = 151	*P*-value
Recipient characteristics				
Male, *N* (%)	123 (62)	34 (69)	89 (59)	0.24
Age, years, mean ± SD	54.8 ± 13.8	57.6 ± 13.2	53.9 ± 14	0.11
BMI, mean ± SD	25.1 ± 4.4	26.4 ± 4.5	24.7 ± 4.3	**0.024**
BMI > 30, *N* (%)	26 (13)	8 (16)	18 (12)	0.43
Requiring RRT before KT, *N* (%)	181 (91)	46 (94)	135 (89)	0.57
Peritoneal dialysis, *N* (%)/ Hemodialysis, *N* (%)	19 (10) /162 (90)	4 (9) /42 (91)	15 (11) /120 (89)	0.79
RRT duration, months, median [IQR]	49 [25–71]	58 [32–77]	47 [22–69]	**0.046**
Diabetes mellitus, *N* (%)	53 (27)	17 (35)	36 (24)	0.14
Hypertension, *N* (%)	181 (91)	45 (92)	136 (90)	>0.99
History of cancer, *N* (%)	28 (14)	8 (16)	20 (13)	0.64
History of cardiovascular disease, *N* (%)	42 (21)	15 (31)	27 (18)	**0.057**
Coronary heart disease, *N* (%)	25 (13)	7 (14)	18 (12)	0.63
Peripheral artery disease, *N* (%)	5 (3)	1 (2)	4 (3)	>0.99
History of stroke, *N* (%)	18 (9)	8 (16)	10 (7)	**0.048**
Initial kidney disease				
Diabetic nephropathy, *N* (%)	31 (16)	9 (18)	22 (15)	0.50
Vascular nephropathy, *N* (%)	18 (9)	3 (6)	15 (10)	0.57
Polycystic kidney disease, *N* (%)	20 (10)	6 (12)	14 (9)	0.59
IgA nephropathy, *N* (%)	15 (8)	5 (10)	10 (7)	0.53
Chronic tubulointerstitial nephropathy, *N* (%)	6 (3)	2 (4)	4 (3)	0.64
Unknown, *N* (%)	49 (25)	10 (20)	39 (26)	0.57
Other, *N* (%)	61 (31)	14 (29)	47 (31)	0.86
KT > 1, *N* (%)	36 (18)	16 (33)	20 (13)	**0.0046**
Albuminaemia in the 7 days before KT, mean ± SD	40.3 ± 5.2	40.5 ± 4.6	40.2 ± 5.4	0.87
CT-scan data				
SMI (cm^2/m^2) for men/women	49.7 ± 8.6/42.3 ± 7.3	46.7 ± 8.5/38.6 ± 7.5	50.9 ± 8.4/43.2 ± 7	/
Muscle density (HU) in men/women	30.6 ± 9/29.7 ± 8.3	20.2 ± 6.1/18.6 ± 6	34.6 ± 6.3/32.4 ± 6.4	/
Donor characteristics				
Living donor, (%)	26 (13)	6 (12)	20 (13)	>0.99
Donor age, mean ± SD	57.6 ± 17	59.2 ± 16.8	57 ± 17.1	0.44
SCr donor, mean ± SD	90 ± 66	78 ± 30	94 ± 73	0.14
ECD, *N* (%)	111 (56)	30 (61)	81 (54)	0.41
Kidney transplant characteristics				
Cold ischaemia, min, median (IQR)	1032 (822–1276)	1060 (909–1200)	1015 (798–1307)	0.73
Specific anti-HLA antibodies, *N* (%)	59/190 (31)	11/47 (23)	48/143 (34)	0.21
Induction immunosuppressive therapy, *N* (%)	198 (99)	49 (100)	149 (99)	>0.99
Antithymocyte globulin, *N* (%)	138/198 (69)	34 (69)	104/149 (70)	>0.99
Anti-IL-2 receptor antibodies, *N* (%)	59/198 (30)	15 (31)	44/149 (30)	>0.99
Maintenance immunosuppressive therapy				
Calcineurin inhibitors, *N* (%)	182 (91)	48 (98)	134 (89)	0.08
Mycophenolic acid (MPA), *N* (%)	165 (83)	36 (73)	129 (85)	0.06
mTOR inhibitors, *N* (%)	32 (16)	12 (24)	20 (13)	0.21
Steroids, *N* (%)	200 (100)	49 (100)	151 (100)	1.00
Delayed graft function, *N* (%)	88 (44)	24 (49)	64 (42)	0.51

BMI, body mass index; ECD, extended-criteria donor; HU, Hounsfield units (density); IQR, interquartile range; KT, kidney transplantation; Ly, lymphocyte; RRT, renal replacement therapy; sCr, serum creatinine concentration; SMI, skeletal muscle mass index.
P-value was calculated between low-muscle-density and normal-muscle-density groups using a χ^2 test for categorial variables and *t*-test for quantitative variables. *P*-values < 0.05 are in bold.

Primary endpoint

Follow-up took place 1322 [1005–1680] days after transplantation. At the end of follow-up and according to the Kaplan–Meier analysis, patient survival was significantly lower in the myosteatosis group than in the normal MD group (*P* = 0.0024) (*Figure* 3A). During follow-up, 14 (29%) low-MD recipients and 21 (14%) normal-MD recipients died. In the myosteatosis group, the causes of death were infectious events (*N* = 7; 50%), cardiovascular events (*N* = 4; 29%), cancers (*N* = 2; 14%), and unknown (*N* = 1; 7%). The causes of death in the normal-MD group were not statistically different and included infectious events (*N* = 9; 43%), cardiovascular events (*N* = 3; 14%), cancers (*N* = 7; 33%), and unknown (*N* = 2; 10%).

Age, myosteatosis, diabetes mellitus status, former cardiovascular events (including coronary heart disease, stroke, and peripheral artery disease), ECD, and lymphopenia $< 1 \times 10^9$/L within 7 days before KT were significantly associated with mortality in univariate analysis (*Table* 3). In multivariable analysis, low MD was an independent risk factor for mortality

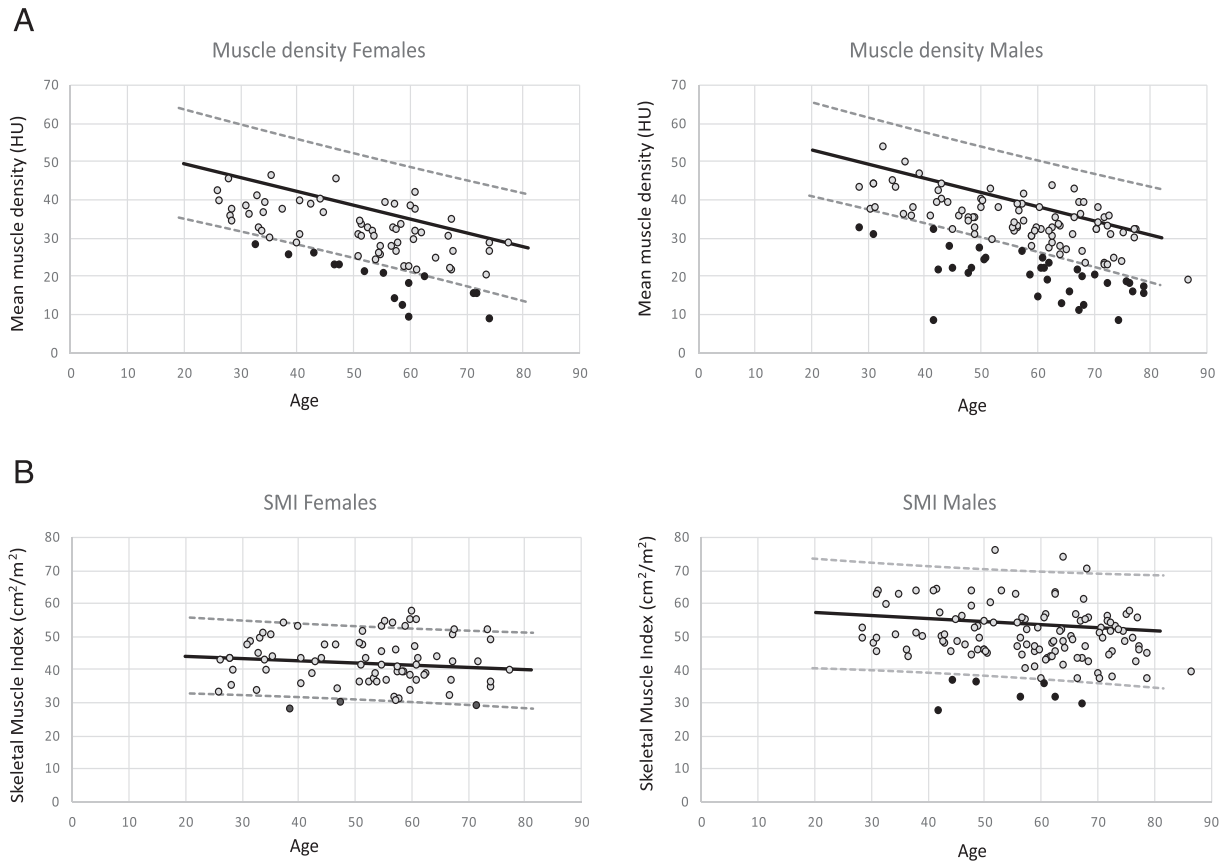

Figure 2 *(A and B)* Association between age and MD *(A)* and between age and SMI *(B)* in women and men. The equations of linear regressions that model association between MD and age were 60.61 − 0.37 × age for men and 56.45 − 0.36 × age for women. The lower limit of the 95% prediction interval were 62.49 − 0.277 × age − 9.28 * (1.02 + (age − 45.14)^2/9426)$^{0.5}$ for men and 61.84 − 0.295 × age − 11.54 * (1.014 + (age − 53.97)^2/11 795)$^{0.5}$ for women. The equations for the association between SMI and age were 58.95 − 0.093 × age for men and 45.5 − 0.072 × age for women. The lower limit of the 95% prediction interval were 45.51 − 0.0719 × age − 10.97 * (1.013 + (age − 53.239)^2/12 954)$^{0.5}$ for men and 58.95 − 0.0928 × age − 15.858 * (1.0185 + (age − 45.154)^2/9991)$^{0.5}$ for women. The black lines represent the linear regression line in healthy subjects, and the dotted lines indicate the 95% prediction intervals. Kidney transplant recipients with an MD or an SMI below the 2.5th percentile for healthy subjects are considered to have myosteatosis or a low muscle mass (black dots). Other subjects are considered to have normal MD and/or normal muscle mass (grey dots). HU, Hounsfield units; MD, muscle density; SMI, skeletal muscle mass index.

after KT [adjusted HR (aHR) 2.12, 95% CI: 1.06–4.24, *P* = 0.033], together with cardiovascular disease (aHR 2.06, 95% CI: 1.02–4.15, *P* = 0.043) and age (aHR 1.06, 95% CI: 1.03–1.09, *P* = 0.0003) (*Table* 3).

Secondary endpoints

Death-censored allograft survival was similar in the low-MD and normal-MD groups of KTr (*Figure* 3B). Delayed graft function proportion, M12 and M24 eGFR, acute rejection rate, community-acquired infection rates, and OI were also similar in the two groups (*Table* 4).

Analysis of the low skeletal muscle mass group

The low-SMI group contained 10 KTr (*Table* S2). These patients were leaner (BMI 20.4 ± 2.8 vs. 25.4 ± 4.4, *P* = 0.0004), had been on dialysis for longer (65 [58–84] months vs. 47 months [24–70], *P* = 0.047), less frequently had hypertension [*N* = 7 (70%) vs. *N* = 174 (92%); *P* = 0.02], and had lower blood albumin levels (37.3 vs. 40.5 g/L, *P* = 0.04).

Primary endpoints

Two of the 10 patients with low SMI (20%) died during follow-up. This frequency was similar to that for KTr with a normal SMI. No graft loss was reported in the low-SMI group. We found that eGFR within 12 months of KT was significantly higher than that in patients with a normal SMI (82 [53–88] mL/min/1.73 m^2 vs. 49 [37–68] mL/min/1.73 m^2, respectively; *P* = 0.028); eGFR values at 24 months were similar. OI incidence was significantly higher [*N* = 3 (30%) vs. *N* = 18 (9%); *P* = 0.039], but there was no difference in acute rejection or community-acquired infection rates between the groups (*Table* S3).

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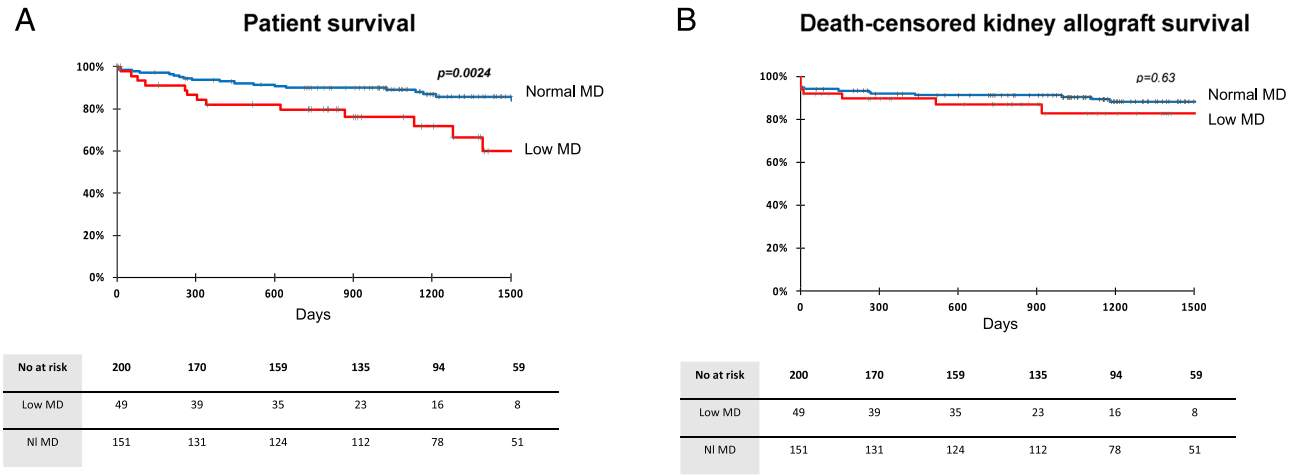

Figure 3 Fi *(A and B)* The Kaplan–Meier curves for patient survival *(A)* and death-censored graft survival *(B)*, in patients with and without myosteatosis. The *P*-value was determined by a log-rank test. Mortality rate was significantly higher in patients with myosteatosis. There was no difference in death-censored graft survival between the groups. MD, muscle density.

Table 2 Association of baseline kidney transplant recipients' characteristics with myosteatosis in univariate and multivariate logistic regression analysis

	Univariate analysis			Multivariate analysis		
Variables	cOR	95% CI	*P*-value	aOR	95% CI	*P*-value
Age (for 1 year increase)	1.02	0.996–1.05	0.11	—	—	—
Diabetes mellitus	1.70	0.85–3.41	0.14	—	—	—
BMI $>$ 25 kg/m^2	1.86	0.97–3.57	0.061	2.94	1.40–6.18	**0.004**
History of stroke	2.75	1.02–7.42	**0.046**	3.7	1.30–10.7	**0.015**
RRT duration (for 1 month increase)	1.01	1.00–1.02	**0.049**	—	—	—
KT $>$ 1	3.18	1.49–6.79	**0.003**	5.2	2.22–12.4	**0.0001**

aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; cOR, crude odds ratio; KT, kidney transplantation; RRT, renal replacement therapy.
ORs were calculated using a univariate then a multivariate logistic regressions analysis. Backward conditional selection procedure, with a 5% exclusion threshold, was applied until the final model was obtained. No variable was forced. In multivariate analysis, variables were adjusted for BMI $>$ 25, KT $>$ 1, and history of stroke. *P*-values $<$ 0.05 are in bold.

Table 3 Univariate and multivariate Cox proportional hazards regression for mortality in kidney transplant recipients

	Univariate analysis			Multivariate analysis		
Variables	cHR	95% CI	*P*-value	aHR	95% CI	*P*-value
Age (for 1 year increase)	1.06	1.03–1.10	$<$**0.0001**	1.06	1.03–1.09	**0.0003**
Myosteatosis	2.77	1.40–5.50	**0.004**	2.12	1.06–4.24	**0.033**
Sex (male)	2.18	0.99–4.81	0.053	—	—	—
Diabetes mellitus	2.75	1.41–5.36	**0.003**	—	—	—
History of cancer	2.08	0.97–4.46	0.059	—	—	—
History of CVD	2.73	1.37–5.44	**0.004**	2.06	1.02–4.15	**0.043**
KT $>$ 1	1.74	0.81–3.71	0.15	—	—	—
ECD	3.71	1.62–8.49	**0.002**	—	—	—
Ly $<$ 1000/mm^3	2.52	1.29–4.91	**0.007**	—	—	—

aHR, adjusted hazard ratio; cHR, crude hazard ratio; CI, confidence interval; CVD, cardiovascular disease (composite criterion including coronary heart disease, stroke, and peripheral arterial disease); ECD, extended-criteria donor; KT, kidney transplantation; Ly, lowest lymphocyte level during the 7 days before kidney transplantation.
P-value was calculated using a univariate then a multivariate Cox proportional hazards regression analysis. Backward conditional selection procedure, with a 5% exclusion threshold, was applied until the final model was obtained. No variable was forced. In multivariate analysis, variables were adjusted for age, myosteatosis status, and history of cardiovascular disease. *P*-values $<$ 0.05 are in bold.

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Table 4 Outcomes after kidney transplantation, by myosteatosis status, as defined by computed tomography scan

Variables	Whole cohort, *N* = 200	Low muscle density, *N* = 49	Normal muscle density, *N* = 151	*P*-value
Delayed graft function, *N* (%)	88 (44)	24 (49)	64 (42)	0.51
M12 eGFR median [IQR]	49 (37–69)	48 (39–66)	49 (37–70)	0.94
M12 DSA, *N* (%)	58/161 (36)	9/35 (26)	49/126 (39)	0.17
M24 eGFR, median [IQR]	45 (33–66)	45 (39–72)	45 (32–65)	0.43
M24 DSA, *N* (%)	43/140 (31)	6/29 (21)	37/111 (33)	0.26
Time from KT to LFU, days, median (IQR)	1322 (1005–1680)	1011 (799–1403)	1368 (1055–1747)	**0.0022**
LFU eGFR, median [IQR]	42 (27–57)	41 (28–56)	42 (27–57)	0.76
Acute rejection, *N* (%)	37 (19)	10 (20)	27 (18)	0.68
Infections, *N* (%)	103 (52)	26 (53)	77 (51)	0.87
Community-acquired infections, *N* (%)	97 (49)	24 (49)	73 (48)	0.94
Opportunistic infections, *N* (%)	21 (11)	8 (16)	13 (9)	0.16
BK viraemia, *N* (%)	29 (15)	5 (10)	24 (16)	0.48
BK virus nephropathy, *N* (%)	9/29 (31)	2/5 (40)	7/24 (29)	0.63
Death, *N* (%)	35 (18)	14 (29)	21 (14)	**0.029**
Infectious disease, *N* (%)	16/35 (46)	7/14 (50)	9/21 (43)	0.68
Cardiovascular, *N* (%)	7/35 (20)	4/14 (29)	3/21 (14)	0.31
Cancer, *N* (%)	9/35 (26)	2/14 (14)	7/21 (33)	0.21
Unknown, *N* (%)	3/35 (9)	1/14 (7)	2/21 (10)	0.81
Allograft loss, *N* (%)	27 (14)	7 (14)	20 (13)	0.81

DSA, donor-specific antibodies; eGFR, estimated glomerular filtration rate; IQR, interquartile range; KT, kidney transplantation; LFU, last follow-up.
P-value was calculated between low-muscle-density and normal-muscle-density groups using a χ^2 test for categorial variables and *t*-test for quantitative variables. *P*-values < 0.05 are in bold.

Discussion

This retrospective study of 200 patients evaluates risk factors for and consequences of low skeletal muscle mass and myosteatosis in KTr. Our findings indicate that myosteatosis, acquired during the pre-transplant period, is both highly prevalent in our population and an independent risk factor for death.

This study is the first to describe the prevalence of myosteatosis in patients undergoing KT. A quarter of our population of KTr had an MD below the expected 2.5th percentile for age and sex, whereas only 5% of patients had an SMI below the 2.5th percentile. A higher prevalence (up to 50%) of myosteatosis was reported in a population of liver transplant recipients.[24] However, the thresholds in this previous study were not adjusted for age, whereas we used an age-adjusted definition of myosteatosis because we observed a strong association between age and myosteatosis in healthy subjects.

The different pre-existing low-MD cut-offs established in the literature are not adapted to our population. Indeed, using the same cut-off points for the definition of myosteatosis as used in the liver transplant cohort and in previous studies[13,25] (i.e. a mean MD $<$ 41 HU for patients with a BMI up to 24.9 kg/m^2 and $<$33 HU for patients with a BMI $\geq$ 25 kg/m^2), up to 90% (*N* = 171/200) of KTr would have been considered to have myosteatosis.

There is some evidence to suggest that myosteatosis is linked to ESRD. Keddar *et al*. showed, in a cohort of patients undergoing peritoneal dialysis, that (i) myosteatosis was associated with the volume of residual diuresis, (ii) fat progressively accumulated in muscle in parallel with the deterioration of renal function, and (iii) MD was generally lower than in subjects with normal renal function.[11] The pathophysiological processes leading to myosteatosis during CKD remain unclear but may involve age, chronic inflammation,[26] and insulin resistance, which is known to be a cornerstone of the pathophysiology of myosteatosis in other contexts.[27] Advanced CKD may cause insulin resistance directly[28] or through the associated metabolic acidosis.[29]

We demonstrated that the main factors conferring a predisposition to myosteatosis in KTr were previous KT, a history of stroke, and a BMI $>$ 25 kg/m^2. This association between previous KT and myosteatosis could be explained by drugs received within previous transplants. Corticosteroids and calcineurin inhibitors promote insulin resistance, and calcineurin inhibitors commonly cause metabolic acidosis.[30] A link between myosteatosis and a history of stroke has been reported before: paretic thigh muscles are both atrophic and prone to fat infiltration.[31] Age, a classic risk factor for myosteatosis,[9,32] was not identified as a predisposing factor in our study because our diagnostic criterion for myosteatosis was age adjusted. Nevertheless, we clearly demonstrated a decrease in MD with age, in our cohort of interest, as in subjects without kidney disease. The duration of dialysis was not an independent risk factor for myosteatosis. We can therefore hypothesize that dialysis quality, rather than the total duration of dialysis, and defects in the control of hydroelectrolytic disorders, including acid–base balance, underlie myosteatosis.

Importantly, we provide the first demonstration in a large cohort of KTr that myosteatosis is an independent risk factor

of mortality after KT. Moreover, our findings confirm known risk factors for mortality after KT, such as age and history of cardiovascular disease.[4] Myosteatosis is known to be associated with mortality in several other contexts, including cancer,[12] liver transplantation,[13] mechanical ventilation in intensive care,[14] and even recently, in a large cohort of healthy people without cardiovascular disease followed up over a period of 11 years.[33] Surprisingly, the frequency of cardiovascular death was not higher in the myosteatosis group. Myosteatosis is associated with mitochondrial dysfunction and chronic inflammatory syndrome, which can increase cardiometabolic risk.[34] The small number of events in our cohort may be responsible for statistical power loss and might explain for the lack of association.

The prevalence of low skeletal muscular mass (SMI) was low in our population of KTr.[17] These results run contrary to previous findings. Most studies have reported a prevalence of low muscle mass of 25–35% in dialysis patients.[5,35] The variation of the values obtained may be explained by both the techniques used to assess muscle mass and the cut-off points selected[23] and emphasizes the need to develop guidelines for the assessment of sarcopenia in the specific population of ESRD patients, as has been done in patients with chronic liver disease.[36] Caution is required when drawing conclusions about risk factors for mortality, due to the small number of events. Former studies have already identified sarcopenia as a risk factor for mortality in the contexts of dialysis[37] and KT[7] on the basis of serum creatinine concentrations at the time of transplantation to assess total muscle mass. Recently, Deliège *et al*. highlighted the association between low muscle mass and increased mortality in older male KTr, using SMI on CT scan at L3 vertebral level as in our study.[8] Moreover, several studies assessed the impact of muscle protein synthesis-related serum biomarkers, such as myostatin on survival for patients with liver cirrhosis[38] and ESRD.[39] Unfortunately, myostatin was not routinely collected in our centre, and we did not have biobank collection. The study of Deliège *et al*. might have some limitations: (i) firstly, it focus only on older KTr (i.e. >60 years old), (ii) the threshold used was the 10th percentile of SMI in there cohort but was not age adjusted and was not based on the SMI from healthy subjects, leading to a less physiological definition than our study, and (iii) SMI was only associated with the combined primary endpoint (i.e. all-cause mortality and/or kidney allograft loss within first year after KT) in men but not in women.[8] The discrepancy between our results and previous findings may also be due to an association of CKD with a loss of muscle predominantly in the appendicular muscles, which would not be picked up by SMI determinations at L3.

Finally, our study has several limitations. Recruitment bias is a potential issue inherent to studies such as this with a retrospective, single-centre design. The size of the sample of healthy subjects (N = 130) used to define the thresholds for normal SMI and MD may also be a weakness of this study. However, SMI values were similar to those obtained in the study of a large population of Caucasian kidney donor candidates by van der Werf *et al*.,[40] but our female and male patients had a lower MD than those in the Dutch study, except for older men (for whom the values obtained in the two studies were similar). These differences may be accounted for by differences in CT-scan acquisition parameters (i.e. slice thickness of 3–5 mm in the Dutch study and 1.25 mm in our study).[41] This discrepancy between studies highlights the need to standardize instrumentation for the analysis of MD.

Conclusions

In conclusion, we have shown that myosteatosis, diagnosed on a single unenhanced cross-sectional CT image at L3 at the time of KT, is highly prevalent and is an independent risk factor for mortality within 4 years of KT. Moreover, our findings indicate that myosteatosis is a muscle health parameter complementary to muscle mass in patients with ESRD undergoing KT. Further studies are required to confirm these results and to establish reproducible MD thresholds, based on standardized CT-scan acquisition techniques adapted to the populations studied. Interventional studies should be performed to investigate the potential reversibility of myosteatosis, through physical activity or exercise, for example, and to determine whether the correction of myosteatosis can decrease the risk of mortality in KTr.

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Conflict of interest

All of the authors of this manuscript have no conflicts of interest to disclose.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Healthy subjects characteristics.
Table S2: Patients characteristics according to muscle mass status, as defined by CT scan.
Table S3: Outcomes after KT, according to SMI status, as defined by CT scan.

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