A RETROSPECTIVE ANALYSIS OF THE IMPACT OF SARCOPENIA ON LIVER TRANSPLANTATION OUTCOMES

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DECLARATION

I, Natalie Elizabeth Anne Irwin declare that this research report is my own work. It is being submitted for the degree of Master of Medicine (in the submissible format with my protocol and an extended literature review) in the branch of Internal Medicine at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

5th day of October 2020 in Johannesburg

For my parents,

Beverley and David Irwin

ABSTRACT

Background. Myosteatosis and muscle wasting (sarcopenia) are common complications of cirrhosis. Overweight patients with concurrent muscle depletion have a condition termed sarcopenic obesity. This study describes these muscle abnormalities in adult liver transplantation (LT) recipients and investigates their impact on post-transplantation outcomes.

Methods. Records from 106 LT recipients were retrospectively analysed. Muscle abnormalities were determined on computed tomography using segmentation software. Myosteatosis was measured by mean muscle attenuation and sarcopenia by skeletal muscle index (SMI), determined at the third lumbar vertebra using validated gender- and body mass index (BMI)-specific cut-offs. Sarcopenic obesity was diagnosed in recipients with a BMI $\geq$25kg/m^2 who also met the SMI cut-off for sarcopenia. Primary endpoints were the impact of these muscle abnormalities on patient and graft survival at one year after LT. Secondary outcomes were the impact on postoperative complications and length of hospital stay.

Results. The majority of patients were males (n = 64, 60%) aged 50 to 64 years old (n = 47, 44%). Alcoholic and/or non-alcoholic steatohepatitis were the commonest aetiologies of cirrhosis (n = 38, 36%). Myosteatosis was present in 76 patients (72%), 69 had sarcopenia (65%) and 36 had sarcopenic obesity (34%). Myosteatosis increased the risk of mortality at one year after LT (Hazard ratio (HR) 3.3, 95% confidence interval (CI) 1.00-11.13, p = 0.049). Sarcopenia increased the risk of death, but was of borderline significance (HR 2.64, 95% CI 0.99-7.00, p = 0.051). Patients with myosteatosis were at greater risk of allograft failure (HR 4.1, 95% CI 1.2-13.5, p = 0.021) and tended to have longer hospital stays (21, 95% CI 16-27 vs 16 days, 95% CI 10-20, p = 0.015).

Conclusions. Compared to patients without muscle abnormalities, LT recipients with myosteatosis and/or sarcopenia had an increased risk of death at one year. The risk of death was higher with myosteatosis than sarcopenia. Myosteatosis increased the risk of allograft failure at one year and these patients had a longer median length of hospital stay.

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ABBREVIATIONS

ASH/NASH	Alcoholic and/or non-alcoholic steatohepatitis
BMI	Body mass index
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CT	Computed tomography
HU	Hounsfield units
INR	International normalized ratio
LFT	Liver function test
LT	Liver transplantation
MELD	Model for end-stage liver disease
MRI	Magnetic resonance imaging
SMI	Skeletal muscle index
VATI	Visceral adipose tissue index
WDGMC	Wits Donald Gordon Medical Centre

CHAPTER 1: PROTOCOL AND EXTENDED REVIEW OF THE LITERATURE

1.1 Background

Liver transplantation (LT) is the treatment of choice for patients with end-stage chronic liver disease (1). The dramatically increasing prevalence of non-alcoholic steatohepatitis (NASH), associated with obesity and diabetes, as well as a climbing burden of alcoholic hepatitis, drive the rising incidence of cirrhosis worldwide (2, 3). Furthermore, despite advances in immunization and direct acting antiviral therapy, cirrhosis and hepatocellular carcinoma secondary to viral hepatitis B and C remain major global health problems (2, 4). Accordingly, the number of patients requiring LT is climbing (2, 5).

LT is however a costly procedure and in South Africa, small living donor LT programmes and low deceased donor rates are barriers to more widespread access to this life-saving procedure (6, 7). In response to this critical shortage of donor organs, alternatives to using full sized livers from donors after brain death are increasingly used (5). These alternative procedures include split liver grafts and more recently the use of extended criteria donor grafts, such as those donated after circulatory death or from donors with hepatic steatosis, positive hepatitis C serology or advanced age (2, 5, 8). Favourable outcomes with these so called, marginal grafts are documented, however identification of recipients at greater risk for poor morbidity and mortality outcomes may guide their utility (8, 9).

There is evidence that sarcopenia is of clinical significance in morbidity and mortality outcomes after LT (10-14). The European Working Group on Sarcopenia in Older People

defines the umbrella term sarcopenia as a muscle disease characterised by low muscle strength with low muscle quantity or quality. The group further stratify sarcopenia severity according to physical performance in active dynamic tests (15). Both low quantity wasted muscle (sarcopenia) and low quality fat-infiltrated muscle (myosteatosis) are frequent complications of cirrhosis (10, 16). Further investigation on the clinical relevance of these body composition measures on LT outcomes is necessary.

1.2 Literature review

1.2.1 Liver transplantation in South Africa and abroad

Investigation on LT outcomes in South Africa is growing. Two adult liver transplant centres are registered in the country, one in Cape Town at Groote Schuur Hospital and one in Johannesburg at Wits Donald Gordon Medical Centre (WDGMC). The number of transplants performed in each of these programmes has increased exponentially since their inception (6, 17, 18). A review of paediatric and adult LTs performed at Groote Schuur Hospital between 1988 and 2000, evaluated survival outcomes between two months and 9.5 years post-transplantation. Patient survival at the time of review was 61.7% and the cumulative graft survival rate at five years was 61% (17). The more recently audited adult orthotopic LT programme at WDGMC, showed a recipient survival rate at 90 days, one year and five years of 87.6%, 81.7% and 71.0% respectively for transplantations performed between 2004 and 2016. Graft survival at 90 days was 85.8% and 81.0% at one year. Sepsis was the most common cause of death in the first 90 days after transplantation (6).

International transplant centres report comparable data. The Organ Procurement and Transplantation Network publishes an annual report on LTs performed in the United States of America. For deceased donor transplantations performed in 2017, graft survival at one year was 91.2% (2). Analysis of LTs from the European Liver Transplant Registry documents a current patient survival rate of 83% at one year. Morbidity and mortality in this population is greatest in the first year after transplantation. In the first six months, this is largely secondary to sepsis or surgical complications (5). However, globally graft and patient survival rates are improving. This is attributed to advances in donor and recipient selection, intra-operative surgical technique and post-operative immunosuppression (2, 5, 6).

1.2.2 Sarcopenia and cirrhosis

Sarcopenia is common amongst recipients before and after LT (14, 19). Depending on the definition used, prevalence in patients with cirrhosis ranges from 30 to 70% (20, 21). It is more common in males than females (10, 22) and more commonly seen in alcoholic liver disease, than cirrhosis of other aetiology (13). Amongst a prospectively studied recipient population, Tsien *et al.* describe that 75% of study participants with normal muscle mass preoperatively, developed new onset sarcopenia after transplantation (19). The disease is generally underdiagnosed and has not yet been described in the South African transplantation population. Pre-operative sarcopenia in recipients has been identified as a risk factor for morbidity, mortality and poor quality of life in multiple studies (10-14, 22, 23). This includes an increased risk of sepsis (12, 22), longer duration of intubation (13), longer length of stay in intensive care (13) and longer total length of stay in hospital (13,

22). The impact of sarcopenia on survival is independent of Model for End-Stage Liver Disease (MELD) score and Child-Turcotte-Pugh classification (Appendices A and B) (24).

The measurement of sarcopenia prior to transplantation consequently appears to represent an objective indicator of prognosis. The European Working Group on Sarcopenia in Older People presents a comprehensive and updated definition of sarcopenia intended for clinical use in geriatrics: a muscle disease characterised by low muscle strength with low muscle quantity or quality. The group delineates primary (age-related) and secondary (systemic disease related) sarcopenia and describes three parameters as a diagnostic algorithm, shown in Table 1.1 (15). However, thus far much of the literature on sarcopenia in the LT setting is unidimensional, focusing solely on criterion 2 of this operational definition. There is less data on muscle strength and physical performance and much heterogeneity in the definition and technique of measurement of muscle quantity.

Table 1.1 European Working Group on Sarcopenia in Older People 2018 operational definition of sarcopenia (15)

Probable sarcopenia is identified by criterion 1. **Diagnosis is confirmed by additional documentation of criterion 2.** **If criteria 1, 2 and 3 are all met, sarcopenia is considered severe.**
Criterion 1. Low muscle strength Criterion 2. Low muscle quantity or quality Criterion 3. Low physical performance

1.2.3 Muscle strength

Muscle strength is most often measured by hand grip using a calibrated handheld dynamometer. It is easy and cost-effective to perform, has been validated and correlates excellently with many outcome measures in geriatric populations (15). Accordingly, The European Working Group on Sarcopenia in Older People regards low muscle strength as a pivotal entry-point criterion in the diagnosis of sarcopenia (15). In the LT setting, Daphnee *et al.* assessed handgrip in patients on the waiting list and report that low grip strength is a strong predictor of mortality (25).

1.2.4 Muscle quantity

Comparative analysis of sarcopenia in LT, is limited by the lack of a standardized technique to measure muscle quantity. Computed tomography (CT) and magnetic resonance imaging (MRI) are the gold standard investigations for quantifying muscle mass in research settings as they are precise and can distinguish muscle from fat (26). Recipients often routinely undergo abdominal CT scans in the pre-operative period, so images should be readily available without re-exposure to radiation. Bioelectrical impedance analysis and dual-energy X-ray absorptiometry are both accurate techniques for the assessment of total body composition and have been used in both clinical and research settings. They are safe

and relatively inexpensive and both have well validated age-, gender- and race-specific reference values defining sarcopenia (15, 26). However, bioelectrical impedance analysis is not accurate when there is fluid retention and so is of limited use in patients with end-stage liver disease (27). Giusto *et al.* document poor correlation between dual-energy X-ray absorptiometry and the gold standard CT measure (28). Likewise, body mass index (BMI) and typical anthropometric measurements, as detailed in Table 1.2, correlate poorly with the degree of sarcopenia, particularly in patients with chronic liver disease. This is attributable to the changes in body composition associated with end-stage disease, such as ascites and loss of adipose tissue (29, 30).

Table 1.2 Body size and composition measures in chronic liver disease

Method	Measurement	Weaknesses in the setting of chronic liver disease
BMI	Mass (kg)/ Height $(m)^2$	Increased total body water results in overestimation of BMI (21)
		Does not measure body composition. No discrimination between lean muscle and adipose tissue
		Sarcopenia is not only diagnosed in patients with low BMI (16, 21)
Anthropometry	Mid-arm muscle circumference, skin fold thickness or calf circumference	Low sensitivity as subcutaneous fat or oedema results in overestimation of lean muscle mass (29)
		Poor correlation between mid-arm muscle circumference and the gold standard CT measure of sarcopenia (28)

In LT research, most commonly, a single CT cross-sectional measure of either the total skeletal muscle area or the area of the psoas muscle alone is used as a correlate of muscle mass. There is considerable heterogeneity in the definition and technique of measurement across studies. More commonly described is the total skeletal muscle area. This can be quantified using automated segmentation software set to tissue specific Hounsfield unit thresholds (as shown in Figure 1.1). In much of the literature on patients with cirrhosis, -29 to +150 Hounsfield units are the chosen thresholds for muscle. This avoids over-estimation of the muscle area in the presence of ascitic fluid. The area is then normalized for height, computing a value known as the skeletal muscle index (SMI) measured in cm^2/m^2 (31). Correlation between various segmentation software programmes has been proven (32). The North American Working Group on Sarcopenia in Liver Transplantation emphasises that SMI measured on CT scan is the most well-researched technique for assessing low muscle mass in patients with cirrhosis (33).

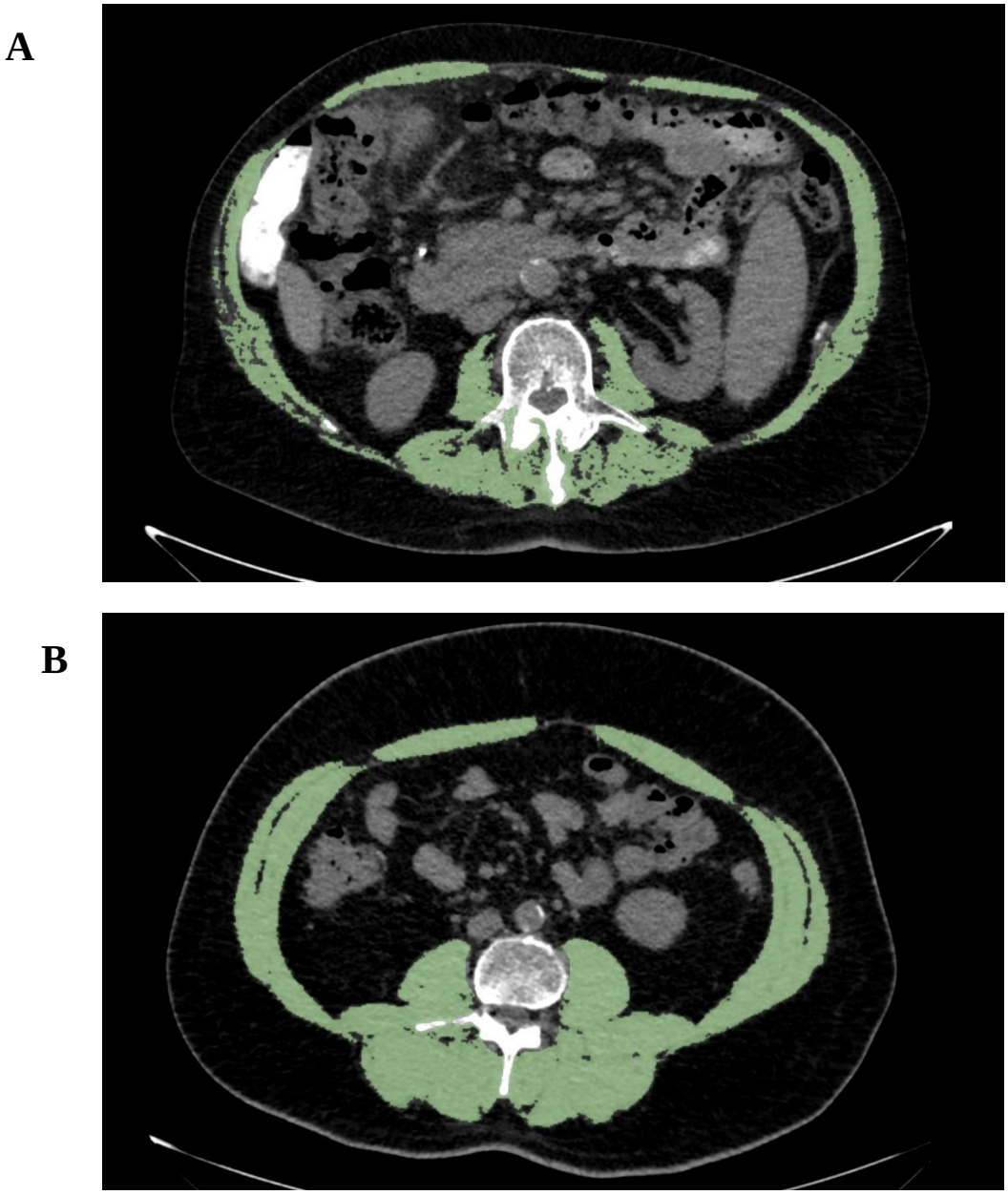

Figure 1.1 Skeletal muscle index quantification using segmentation software. Figure A and B show the abdominal CT scans of two male patients with cirrhosis and identical BMIs ($32kg/m^2$) analysed in this current research report. Patient A is sarcopenic (SMI L3 = $39cm^2/m^2$) and patient B has normal muscle quantity (SMI L3 = $61cm^2/m^2$) (34).

The cross-sectional level of the third lumbar vertebra is typically used as a reference point when computing the SMI. At this level, the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal oblique, and rectus abdominis muscles are visualized (31). The choice of the third lumbar vertebral level seems to be heavily based

on the findings of a study published by Shen *et al.* in 2004. This team investigated the relationship between abdominal skeletal muscle area, measured on a single transverse MRI slice, and the total body skeletal muscle volume, measured on whole body MRI. Amongst their population of 328 healthy and ethnically diverse adults, single skeletal muscle slice areas correlated best with total body skeletal muscle volumes at approximately 5cm above the fourth/ fifth lumbar intervertebral space (35). Mourtzakis *et al*. present further data in support of the third lumbar vertebra. In their study, SMI at the third lumbar vertebra, correlated well with whole body skeletal muscle mass measured on Dual-energy X-ray absorptiometry in 51 patients with colorectal or lung cancer (31). The muscle area measured at the third lumbar vertebral level is consequently largely accepted amongst researchers as a good correlate of total body skeletal muscle. However, alternative levels such as the fourth lumbar vertebra (19), the level of the umbilicus (36) or a level within the thigh region (35) have been proposed in studies but need further validation.

Some studies use the psoas muscle alone rather than the SMI at the third lumbar vertebra. Several measures to define sarcopenia with this muscle are proposed including the psoas muscle area, psoas muscle area normalized for height, transverse psoas muscle thickness normalized for height and psoas muscle volume normalized for height. Each have been identified as a poor prognostic factor for complications or mortality after LT (12, 14, 36-38). The rationale for using the psoas muscle is not clearly described. Some suggest it is chosen as the muscle is easily identifiable on CT scan or that as a deep hip flexor, it may be a good surrogate of muscle loss (39). There is no standardized definition of these above measures, nor an agreed upon technique to quantify them. Nevertheless, psoas muscle measurements appeal as they are simple and quick and may be more accurate than SMI in the setting of ascites. Data from Golse *et al.* analyses survival in LT recipients and

supports this. Psoas muscle area at the third lumbar vertebra was as accurate as psoas muscle area normalized for height and more accurate than SMI in predicting one-year patient survival (38). Conversely, amongst a population on the LT waiting list, Ebadi *et al.* describe that the psoas muscle index (bilateral psoas muscle area divided by height squared) poorly identifies those at increased risk of mortality when compared to SMI (39). Psoas muscle measurements have not been evaluated as a marker of total body muscle mass.

The matter is further complicated by a lack of standardised age-, gender- and race-specific normal values for both SMI and the psoas muscle measurements. However, efforts to develop a congruous operational definition of sarcopenia in the LT population, are ever advancing. There is recognition that this is needed in order to generate comparable data. Previously a cut-off point of more than two standard deviations below the mean reference values of healthy young adults was recommended by the European Working Group on Sarcopenia in Older People (26). However skeletal muscle indices in the healthy young adult population are poorly studied, so this recommendation is vague. Some studies therefore borrow optimal cut-off values for SMI from the oncology literature. These values have been determined using optimal stratification statistical analysis in diseased populations (40). More recently in an effort to refine the values for the LT population, Carey *et al*. conducted a multicentre study amongst 396 mostly male Caucasian patients in North America. They conclude that in men, a threshold SMI of <50 cm^2/m^2 and in women <39 cm^2/m^2 correlates best with mortality on the LT waiting list (41). In 2019 the North American Working Group on Sarcopenia in Liver Transplantation released an expert opinion statement advocating these sex-specific cut-off values (33). Similar studies have not yet been conducted elsewhere in the world and so these values will require validation

in different populations and in a larger female cohort. Standardized cut-off values for the psoas muscle indices have not been studied.

1.2.5 Muscle quality: myosteatosis and sarcopenic obesity

Interest in the significance of intramuscular fat in LT recipients is growing as sarcopenia can be associated with either preserved or increased intramuscular fat deposition. The radiodensity of a muscle, measured in Hounsfield units on CT scan analysis is a measure of its fat content. Low muscle radiodensity, also termed low mean muscle radiation attenuation (MRA) or myosteatosis is a common and poor prognostic factor for late and early mortality in LT patients (16, 42). It is associated with higher risk of hepatic encephalopathy, higher post-operative complication rates, longer length of stay in intensive care, longer overall hospital stay and higher costs (43, 44). In many studies the incidence of myosteatosis is higher than that of sarcopenia (16, 42-44). There is no universal consensus on the measurement technique, nor defining cut-off value for myosteatosis (15). Once again, some authors use threshold values associated with lower survival which are derived from the oncology literature. Specifically, in these populations, <41 Hounsfield units in patients with a BMI <24.9kg/m^2, and <33 Hounsfield units in those with a BMI ≥25kg/m^2 define myosteatosis (45).

The term sarcopenic obesity is used to describe patients with both loss of lean muscle mass and excess deposition of intermuscular and intramuscular adipose tissue. Although easily clinically overlooked, sarcopenic obesity has been described in up to 40% of LT recipients on CT based muscularity assessment (46). It is diagnosed in patients who are both obese or overweight (BMI ≥25kg/m^2) and meet SMI criteria for sarcopenia. As the global incidence

of obesity with non-alcoholic fatty liver disease grows, the prevalence of overweight patients on the LT waiting list can be expected to grow (3). Obesity begets both sarcopenia and myosteatosis (16, 26). Sarcopenic obesity is significantly associated with higher perioperative risk as patients are at risk of the complications of both obesity and sarcopenia (26). In cognisance of this, The North American Working Group on Sarcopenia in Liver Transplantation notes than in future research, objective, image-based measures of obesity, which are superior to BMI will be required (33).

1.2.6 Physical performance

Physical performance is the third parameter of the European Working Group on Sarcopenia in Older People's consensus definition. It is usually measured by active dynamic tests such as the gait speed, the Timed-Up and Go test or the Short Physical Performance Battery test. Assessment of physical performance is used to grade severity of sarcopenia as described in Table 1.1 (15). Studies have validated many of these active dynamic tests and they show good correlation with measures of skeletal muscle in patients with end-stage liver disease (47). However, the North American Working Group on Sarcopenia in Liver Transplantation highlights the limitations of hand grip and active dynamic tests in the acutely ill and patients with cognitive deficits. They note that muscle mass is more objective and chose not to incorporate measures of muscle strength or physical performance in their definition of sarcopenia (33).

Instead, the term frailty, a concept more typically associated with geriatrics, is fast becoming an area of interest in LT research. It is described as a distinct clinical syndrome characterised by cumulative deterioration in physical, cognitive and social functioning (15,

48). Sarcopenia, the disease, is delineated from the broad frailty syndrome and from cachexia, a complex metabolic syndrome of muscle and fat loss associated with underlying disease. Rather, sarcopenia exacerbates the development of physical frailty (15, 26). The frailty syndrome is diagnosed using multidimensional frailty measurement tools which include the measures of muscle strength and physical performance described above. Data reflect that frailty is common amongst patients before LT owing to dysfunctional protein synthesis, malnutrition, physical inactivity and sarcopenia (48). Lai *et al.* constructed a frailty index including grip strength, chair stands and balance assessment and report that this metric accurately predicted waitlist mortality in patients with cirrhosis (49). The frailty syndrome persists and, in some cases, worsens after LT. There is legitimate concern that this will negatively impact long and short-term outcomes (48).

1.2.7 Visceral fat

The area of adipose tissue on a single transverse CT slice is also proposed as a relevant image-based measure of obesity in LT recipients. In the same way that the cross-sectional area of muscle is quantified, the area of fat is quantified using segmentation software set to the specific Hounsfield units for adipose tissue (-150 to -50 for visceral fat and -190 to -30 for subcutaneous fat) (50, 51). Standardized cut-off values defining visceral obesity in the LT population are not yet defined. In a retrospective study of deceased-donor LT recipients, Terjimanian *et al*. describe that increased abdominal visceral fat is significantly associated with worse survival outcomes at both one and five years after LT (52). Abdominal adiposity has similarly been shown to increase the risk of recurrence of hepatocellular carcinoma in male patients with cirrhosis after LT (53). Ebadi *et al*. describe that body composition has differing impacts on prognosis in males and females with

cirrhosis. In their study, low subcutaneous fat was associated with mortality in female, but not male patients. Low skeletal muscle index predicted mortality in male, but not female patients and in contrast to results from Terjimanian *et al.*, visceral fat was not associated with survival (54). Other authors highlight that the complex endocrine and paracrine action of fat varies depending on its distribution and therefore suggest that the ratio of visceral to subcutaneous fat may prove a more reliable predictor of outcome (42).

1.2.8 Body composition and the MELD score: The way forward?

Despite the use of extended donor criteria, traditional cadaver liver grafts are a scarce and costly resource. The development of ethically just, as well as clinically sound organ allocation policies is of fundamental importance in maximising medical utility of LT services. The now ubiquitous MELD score, which prioritises transplant waiting list candidates, was a key advancement in these allocation policies. It was adopted by the United Network for Organ Sharing in 2002 and has been validated by numerous studies (55, 56). Three objective variables namely, serum total bilirubin, serum creatinine and the international normalized ratio of prothrombin time (INR) are used to calculate a score between six and 40. This score reliably predicts three month mortality in patients with chronic liver disease (55). Previously, organ allocation policies relied on ranking according to waiting time on the list and the more subjective Child-Turcotte-Pugh score. Data reflect that in the post-MELD era, liver allocation has improved. In the first year that the score was implemented in the United States of America, transplantation rates increased 10 percent and waiting list mortality decreased (57).

Modifications to the MELD score have been proposed to minimize its limitations and improve prognostication, thus improving ranking of candidates on the waiting list. Most notably, the MELDNa score includes serum sodium level. Hyponatraemia is an independent risk factor for mortality in patients with cirrhosis and since January 2016, the Organ Procurement and Transplantation Network recommends that the MELDNa score be used in organ allocation policies (58, 59).

There is increasing postulation that a measure of nutritional status should supplement the MELDNa score in future organ allocation policies. Durand *et al.* measured transverse psoas muscle thickness at the level of the umbilicus and combined it with the MELD score, generating the MELD-psoas score. The discriminative performance of this score for waiting list mortality in patients with cirrhosis was better than the MELD score (36). Montano-Loza *et al.* tested a similar hypothesis, but assessed sarcopenia, measured by the cross-sectional area of skeletal muscle at the third lumbar vertebra. Their study reports that including sarcopenia in the MELD score, improves prediction of mortality, particularly in recipients with low MELD scores (60). However, in a Dutch cohort, the MELD-sarcopenia score failed to have any discriminative value over the MELD score in predicting waiting list mortality (61). A novel Muscle Model for End-stage Liver Disease score (Muscle-MELD) is also proposed, in which both muscle quantity and quality are assessed. When analysed with post-transplantation outcomes, the Muscle-MELD score showed good discriminative performance in predicting mortality at three, six and 12 months after living donor LT (62). These modifications all require validation.

Ongoing research on the impact of sarcopenia on outcomes will ensure that LT allocation policies are guided by evolving and evidence-based clinical knowledge. Consequently, I aim to describe sarcopenia in a South African LT recipient population using previously described CT measurements. I will evaluate the impact, if any, of these measures on post-transplantation morbidity and mortality outcomes.

1.3 Objectives

1. To describe the prevalence of sarcopenia using conventional CT measurements at three axial levels and then determine if any of these measurements are predictive of outcome after LT.
2. To determine the impact, if any, of sarcopenia, sarcopenic obesity, myosteatosis and visceral fat on the following LT outcomes:
    a. Morbidity outcomes measured by post-operative complications and length of hospital stay
    b. Mortality outcomes measured by patient survival rate and graft survival rate at 90 days and one year following transplantation.

1.4 Methods

1.4.1 Study design

Orthotopic adult LTs performed by the University of the Witwatersrand Liver Transplantation Centre at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and WDGMC Liver Transplant Unit from 1 January 2005 to 30 April 2017 will be retrospectively analysed.

1.4.2 Study population

All adult LT recipients (≥18 years old at the time of transplantation), with chronic liver disease as the indication for surgery and with an abdominal CT scan performed within the six months prior to transplantation will be included in the study.

1.4.3 Population size

Approximately 30 to 40 adult LTs for both acute fulminant and chronic liver failure are performed in this centre a year. The estimated population size is 340 LT recipients.

Addendum May 2020

During data collection, the above study period and inclusion criteria were applied, however, only 47 LT recipients had suitable imaging available for analysis. No imaging taken prior to 2011 was accessible. In order to adequately power the study, a request was made to the University of the Witwatersrand Human Research Ethics Committee to extend the study period up to 31 January 2019 and to include patients who had a CT scan performed within the first month after LT.

1.4.4 Data collection

The required data will be collected from the patient records at CMJAH and accessed from the Adult Liver Transplantation Database at Wits Donald Gordon Liver Transplant Unit. These records, the above database and the electronic database of CT imaging, will be accessed with permission from the chief executive officer of each hospital. Each patient will be allocated a unique study number which will only be accessible to the primary investigator and supervisors. The required CT parameters will be measured using segmentation software.

The following variables, detailed in Table 1.3 will be collected. They are defined further in Appendix C and listed in the data collection sheets in Appendix D.

Table 1.3 Pre-transplantation, computed tomography parameters, surgical and post-transplantation outcome data to be collected

Pre-transplantation recipient variables	Age at time of LT
	Sex
	Self-reported race
	Cause of underlying chronic liver disease (indication for LT)
	Height and weight at time of LT to calculate BMI
	Serum creatinine, total bilirubin, international normalized ratio of prothrombin time (INR) and serum sodium level to calculate the MELD/MELDNa scores
	Diabetes mellitus
CT scan parameters	Cross-sectional area of muscle
	Skeletal muscle index
	Intramuscular fat
	Cross-sectional area of visceral fat
	Visceral adipose tissue index
	Bilateral psoas muscle area
	Psoas muscle index
	Psoas muscle thickness
	Psoas muscle thickness normalized for height
Surgical variables	Living donor or cadaver donor
	Donor age
	Graft type and weight
	Duration of cold ischemic time
	Operative time
Post-transplantation outcomes	Duration of hospital stay in days defined as the number of days from transplantation to discharge or death
	Complications (surgical re-exploration, biliary or vascular complications)
	Patient survival at 90 days and one year after LT
	Graft survival at 90 days and one year after LT defined by the absence of graft failure from any cause, including death or the need for re-transplantation

1.4.5 Data analysis

Data will be analysed using SAS version 9.4 (SAS Institute, USA). Continuous data will be described using mean, median and range. Mean ± standard deviation will be used to describe parametric data and the median and interquartile range to describe non-parametric data. The Fisher's exact test will be used to compare categorical variables. A p-value

<0.05 will be considered statistically significant. Missing data will be handled uniformly. Univariate and multivariate analyses will be performed using Cox's regression models. Variables with significant associations after univariate analyses will be included in the multivariate analysis.

1.4.6 Bias

The collection will be standardized for all participants and measurement of CT scan parameters will be blinded to outcome. Multi-variable analysis will be used to minimise the effect of confounding variables. The confounding variables are age, sex, self-reported race, cause of underlying chronic liver disease, BMI, MELD score, diabetes mellitus.

1.5 Endpoints of study

On completion of this study, the relationship between pre-transplantation variables and sarcopenia will be analysed to evaluate the impact (if any) of sarcopenia on morbidity and mortality outcomes after LT.

1.6 Ethics

This proposal will be submitted to the University of the Witwatersrand Human Research Ethics Committee. The intended study is retrospective and anonymous. Permission to access and record the data will be obtained from the chief executive officers of CMJAH and WDGMC. Once compiled, the data will remain in the primary investigator's possession.

1.7 Timing

The study will commence in May 2018 and anticipated duration to completion is 12 months.

Proposed schedule for completion of study

	February 2018	March 2018	April 2018	May 2018	June 2018	July 2018	August 2018	September 2018	October 2018	November 2018	December 2018
Ethics application											
Assessment of protocol											
Collection of data											
Data analysis											
Write up dissertation											
Review of dissertation											
Submit dissertation											

1.8 Funding

Foreseen expenses include only photocopying and binding. This will be self-funded.

1.9 Problems

There may be difficulties tracing and accessing the CT scans for all study participants. Close liaison with the radiology unit will be necessary. This study will be limited by the lack of standardized population specific cut-off values defining the various measures of sarcopenia, myosteatosis and visceral fat in LT recipients. The definitions selected for use are evidence based and valid, but are neither specific to the study population nor the disease spectrum.

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CHAPTER 2: PROPOSED MANUSCRIPT

This proposed manuscript for *Transplantation* is prepared according to the journal's instructions and guidelines for authors using a subset of all the collected data.

The results of data collected, but not included in this proposed manuscript are detailed and analysed briefly in Appendix E.

A RETROSPECTIVE ANALYSIS OF THE IMPACT OF SARCOPENIA ON LIVER TRANSPLANTATION OUTCOMES

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N.E.A.I. participated in research design, data acquisition, data analysis, writing the paper and critical revision. L.L. participated in data acquisition. J.F., K.R.H., A.M., and J.F.B. participated in research design, data analysis and critical revision.

The authors declare no conflicts of interest.

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ABBREVIATIONS

ASH/NASH	Alcoholic and/or Non-alcoholic steatohepatitis
BMI	Body mass index
CT	Computed tomography
LT	Liver transplantation
MELD	Model for end-stage liver disease
MRI	Magnetic resonance imaging
SMI	Skeletal muscle index
WDGMC	Wits Donald Gordon Medical Centre

ABSTRACT

Background. Myosteatosis and muscle wasting (sarcopenia) are common complications of cirrhosis. Overweight patients with concurrent muscle depletion have a condition termed sarcopenic obesity. This study describes these muscle abnormalities in adult liver transplantation (LT) recipients and investigates their impact on post-transplantation outcomes.

Methods. Records from 106 LT recipients were retrospectively analysed. Muscle abnormalities were determined on computed tomography using segmentation software. Myosteatosis was measured by mean muscle attenuation and sarcopenia by skeletal muscle index (SMI), determined at the third lumbar vertebra using validated gender- and body mass index (BMI)-specific cut-offs. Sarcopenic obesity was diagnosed in recipients with a BMI $\geq$25kg/m^2 who also met the SMI cut-off for sarcopenia. Primary endpoints were the impact of these muscle abnormalities on patient and graft survival at one year after LT. Secondary outcomes were the impact on postoperative complications and length of hospital stay.

Results. The majority of patients were males (n = 64, 60%) aged 50 to 64 years old (n = 47, 44%). Alcoholic and/or non-alcoholic steatohepatitis were the commonest aetiologies of cirrhosis (n = 38, 36%). Myosteatosis was present in 76 patients (72%), 69 had sarcopenia (65%) and 36 had sarcopenic obesity (34%). Myosteatosis increased the risk of mortality at one year after LT (Hazard ratio (HR) 3.3, 95% confidence interval (CI) 1.00-11.13, p = 0.049). Sarcopenia increased the risk of death, but was of borderline significance (HR 2.64, 95% CI 0.99-7.00, p = 0.051). Patients with myosteatosis were at greater risk of allograft failure (HR 4.1, 95% CI 1.2-13.5, p = 0.021) and tended to have longer hospital stays (21, 95% CI 16-27 vs 16 days, 95% CI 10-20, p = 0.015).

Conclusions. Compared to patients without muscle abnormalities, LT recipients with myosteatosis and/or sarcopenia had an increased risk of death at one year. The risk of death was higher with myosteatosis than sarcopenia. Myosteatosis increased the risk of allograft failure at one year and these patients had a longer median length of hospital stay.

MAIN BODY TEXT

Introduction

Liver transplantation (LT) is the definitive treatment of choice for patients with end-stage chronic liver disease.[1] Considerable clinical advances in organ preservation, surgical technique, post-operative care and immunosuppression have improved survival outcomes of this life-saving surgery.[2, 3] The procedure is however costly and in South Africa, added barriers to the more widespread implementation of LT are small living donor LT programmes and low deceased donor rates.[4, 5] In this era of organ shortage, further studies on long-term predictors of outcomes after LT may guide difficult clinical decisions regarding organ utility.

Skeletal muscle wasting, termed sarcopenia, is an important predictor of survival in LT recipients.[6] Cirrhosis is frequently associated with malnutrition and muscle wasting and as such both the American Association for the study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) offer guidance on nutritional assessment and management.[7-9] Analysis of muscle quantity on cross-sectional computed tomography (CT) or magnetic resonance imaging (MRI) is a validated and widely available measure of nutritional status in these patients.[10] Currently the best internationally studied measure of sarcopenia in patients with cirrhosis is the skeletal muscle index at the third lumbar vertebra (SMI at L3) on CT.[11] It has been shown to be an independent predictor of mortality, longer length of stay in intensive care and longer total length of stay in hospital.[6, 12]

The term sarcopenic obesity is used to describe patients who are both overweight (body mass index (BMI) $\geq$25kg/m^2) and meet CT SMI criteria for sarcopenia.[12] Sarcopenic obesity has been described in up to 40% of LT recipients and is significantly associated with higher perioperative morbidity, as patients are at risk of the complications of both sarcopenia and obesity.[10, 13]

Recent evidence suggests that not only low quantity skeletal muscle, but also low quality muscle increases the risk of poor outcomes. Myosteatosis, characterised by inter- and intramyocellular fat deposition has emerged as a risk factor for mortality in patients with cancer.[14] Measured in Hounsfield units on cross-sectional CT imaging at L3, low mean muscle radioattenuation reflects the high fat content of muscle. Limited studies in patients with cirrhosis point towards higher post-operative complication rates including a higher risk of hepatic encephalopathy, longer hospital and intensive care unit stays and higher associated costs in patients with myosteatosis when compared to those without.[15, 16] In many studies the incidence of myosteatosis in patients with cirrhosis is higher than that of sarcopenia.[12, 15, 17]

The capacity of CT muscle measurements of nutritional status to predict outcome after LT has not yet been tested in South Africa. This present study describes the prevalence of sarcopenia, sarcopenic obesity and myosteatosis in adult patients undergoing LT, using cross-sectional CT imaging analysis and evaluates the impact of these body composition parameters on patient and graft survival, length of hospital stay and post-operative complications.

Materials and methods

This study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (M180560) and conducted in accordance with the principles of the Declaration of Helsinki of 1996. Patient records were anonymized prior to analysis.

Patients: Deceased-donor adult (age ≥18 years) LTs performed at Wits Donald Gordon Medical Centre (WDGMC) and Charlotte Maxeke Johannesburg Academic Hospital Liver Transplant Unit between 1 January 2011 and 31 January 2019 were assessed. First time LT recipients were included in this retrospective study if they (i) had end-stage liver disease of any aetiology (ii) had a plain abdominal CT scan performed at the discretion of the treating clinician in the six months prior or one month after surgery. During this period, 293 patients underwent LT. Patients with acute liver failure (n = 29), previous liver transplantation (n = 5) or without suitable imaging (n = 153) were excluded. One hundred and six patients were included in the analysis.

Clinical and biochemical data were extracted from the existing WDGMC Adult Liver Transplant Research Electronic Data Capture (REDCap) database. Pre-transplantation variables analysed included demographics (age at time of transplant, sex, self-reported race), BMI, aetiology of chronic liver disease, Model for end-stage liver disease (MELD) score and diabetes. Post-transplantation outcomes included recipient and graft survival at one year, immediate length of stay in hospital defined as the number of days from transplantation to discharge or death and post-operative complications (surgical re-exploration, vascular complications and biliary complications).

Image Analysis and analysed parameters: All CT imaging was obtained with a multidetector CT scanner (Brilliance 64, Philips Medical Systems, Amsterdam, Netherlands).

Unenhanced cross-sectional images were analysed using OsiriX MD segmentation software (Version 10.0.0, Pixmeo SARL, Geneva, Switzerland). The total cross-sectional area of the psoas, erector spinae, quadratus lumborum, transversus abdominus, external and internal oblique and rectus abdominus muscles was quantified at the level of L3 at -29 to 150 Hounsfield units. This area was normalized for height, computing the SMI (cm^2/m^2). Sarcopenia was defined using published cut-off values: SMI in females $<39cm^2/m^2$ and males $<50cm^2/m^2$.[11] Sarcopenic obesity was defined as those recipients with sarcopenia and co-morbid obesity or overweight (BMI $\geq 25kg/m^2$).[12]

Myosteatosis was determined by measuring the mean muscle attenuation for the same muscle area at the L3 level in Hounsfield units and defined according to published cut-off values: mean muscle attenuation <41 Hounsfield units defined myosteatosis if the BMI was $<24.9kg/m^2$ and <33 Hounsfield units if the BMI was $\geq 25kg/m^2$.[18]

A radiologist (L.L.) and a trained observer (N.I.), both blinded to outcome, performed all measurements.

Statistical Analysis: Categorical variables are presented as numbers and percentages. Continuous data are expressed as means ± standard deviations. The chi-squared test or Fisher's exact probability test was used to compare categorical variables. The effect of each study variable on outcome was tested using the Cox proportional hazards regression method and significant variables were then included in a multiple binomial regression model. Cumulative survival curves were determined with the Kaplan-Meier method. Data

were analysed using SAS version 9.4 (SAS Institute, USA). A 5% significance level was used.

Results

Demographics and clinical characteristics of patients evaluated: Of the 106 patients, 64 were male (60%) and the most represented age group was 50 to 64 years of age (n = 47; 44%) and ranged from 20-73 years. By self-reported race, there were 72 Caucasian (68%), 20 Black-African (19%) and 12 Indian (11%) patients. Alcoholic and/or non-alcoholic steatohepatitis (ASH/NASH) were the most common underlying aetiologies of liver disease (38; 36%), followed by autoimmune liver disease (34; 32%). Sixty-one percent of patients were overweight by BMI (>25kg/m^2). Baseline characteristics of all included patients are summarized in Table 2.1.

Eighty-eight percent of the study population had at least one body composition abnormality as presented in Table 2.2. Seventy-six patients had myosteatosis (72%), 69 had sarcopenia (65%) and 36 had sarcopenic obesity (34%). Sarcopenia with co-morbid myosteatosis was diagnosed in 52 patients (49% overall). The mean SMI at L3 was 42 ± 9 cm^2/m^2 and mean muscle attenuation was 32 ± 8 Hounsfield units.

Correlations of skeletal muscle abnormalities with other variables: Muscularity varied significantly by sex. Ninety-one percent of females and 69% of males had myosteatosis ($p < 0.001$). Comparatively, 19% of females and 44% of males had sarcopenic obesity ($p = 0.012$). Sarcopenia was also more prevalent in males, but not significantly (72 vs. 55%, $p = 0.096$). Sarcopenic obesity associated significantly with Caucasian ethnicity compared to Black-African ethnicity (40 vs 10%, $p = 0.040$). In Caucasian, Black-African and

Indian/Asian race groups, the incidence of sarcopenia and myosteatosis was 67/75%, 55/65% and 71/64% respectively, but these differences were not significant ($p = 0.54$ and $p = 0.55$ respectively).

Most patients with sarcopenia, myosteatosis or both were of normal nutritional status by BMI (43% (30/69), 41% (31/76) and 46% (24/52) respectively). Three were underweight (BMI <18.5kg/m^2) and of these, all had both myosteatosis and sarcopenia. The prevalence of sarcopenia decreased with increasing BMI (81% in patients with BMI ≤24.9kg/m^2 vs. 43% in patients with BMI ≥30kg/m^2, $p < 0.01$). There was no significant correlation between myosteatosis and obesity by BMI (72 vs 83%, $p = 0.071$), underlying ASH/NASH aetiology (76 vs. 69%, $p = 0.50$) nor co-morbid diabetes (79 vs. 71%, $p = 0.58$).

Clinical significance of skeletal muscle abnormalities: Median length of stay in hospital censored for death was 18 days (n=89, 95% confidence interval (CI) 15-22 days). Patients with myosteatosis were hospitalized for longer (21 days, 95% CI 16-27 days, $p = 0.015$) than patients without myosteatosis (16 days, 95% CI 10-20 days). Neither sarcopenia nor sarcopenic obesity portended longer median length of hospital stay.

Of the 106 patients, 45 (43%) experienced at least one surgical post-operative complication. Morbidity included 24 patients with a biliary stricture or leak (23%), 21 patients requiring re-exploratory laparotomy (20%) and 19 patients with a vascular complication (18%, most commonly hepatic artery thrombosis in 9/19). No significant associations between myosteatosis ($p = 0.13$), sarcopenia ($p = 0.77$) or sarcopenic obesity ($p = 0.10$) and these complications were found. Patients with ASH/NASH had a lower risk

of post-operative complications (RR 0.51, 95% CI 0.29-0.91, p = 0.023), regardless of muscularity. Categorized MELD score (< or ≥15) did not significantly differ in patients with and without muscle abnormalities.

Survival after LT: Patients with myosteatosis had significantly worse survival at one year in comparison to patients without myosteatosis. [Hazard ratio (HR) 3.3, 95% CI 1.003 – 11.1, p = 0.049, Figure 2.1 A]. In this study, sarcopenia increased the risk of death at one year when compared to those without sarcopenia, but was of borderline significance (HR 2.64, 95% CI 0.99-7.00, p = 0.051, Figure 2.1 B). Overall recipient survival at one year after LT was 82% (95% CI 73-88%). Amongst those who died, sepsis was the most common cause of death (26%, 5/19). Sarcopenic obesity did not significantly increase the risk of death at one year (HR 1.23, 95% CI 0.56-2.71, p = 0.61). For patients with myosteatosis, sarcopenia and sarcopenic obesity, survival rates at one year were 76%, 75% and 81% respectively. All patients without body composition abnormalities were alive at one year after LT compared to 69% of those with co-existing myosteatosis and sarcopenia.

On univariate analysis, few other clinical factors increased the risk for death at one year. Black-African ethnicity non-significantly increased with greater risk of death (HR 2.22, 95% CI 0.93-5.29, p = 0.072). Age, sex, aetiology, BMI, MELD score and diabetes did not influence one-year survival (Supplementary data: Table 2.3).

Myosteatosis significantly increased the risk of allograft failure at one year (HR 4.08, 95% CI 1.24-13.45, p = 0.021), but neither sarcopenia, nor sarcopenic obesity impacted the risk (HR 1.63, 95% CI 0.73-3.67, p = 0.24 and HR 1.12, 95% CI 0.53-2.36, p = 0.76).

Table 2.1 Baseline characteristics of liver transplantation recipients at transplantation

	All patients (n = 106)	**Myosteatosis (n = 76)**	**Sarcopenia (n = 69)**	**Sarcopenic obesity (n = 36)**	**No muscle abnormality (n = 13)**
Age (years)					
18-34	17 (16.0)	12 (15.8)	14 (20.3)	1 (2.8)	1 (7.7)
35-49	30 (28.3)	19 (25.0)	19 (27.5)	8 (22.2)	6 (46.15)
50-64	47 (44.3)	36 (47.4)	28 (40.6)	20 (55.6)	5 (38.46)
≥65	12 (11.3)	9 (11.8)	8 (11.6)	7 (19.4)***	1 (7.7)
Sex					
Male	64 (60.4)	38 (50.0)	46 (66.7)	28 (77.8)**	10 (76.9)
Female	42 (39.6)	38 (50.0)****	23 (33.3)	8 (22.2)	3 (23.1)
Self-reported race					
White	72 (67.9)	54 (71.1)	48 (69.6)	29 (80.5)*	8 (61.5)
Black-African	20 (18.9)	13 (17.1)	11 (15.9)	2 (5.6)	3 (23.1)
Indian/Asian	13 (12.2)	9 (11.8)	9 (13.0)	4 (11.1)	2 (15.4)
Mixed	1 (0.9)	0	1 (1.4)	1 (2.8)	0
Indication for transplantation					
ASH/ NASH	38 (35.8)	29 (38.2)	25 (36.2)	18 (50)*	6 (46.15)
AILD[a]	34 (32.1)	19 (25.0)	16 (23.2)	5 (13.9)	1 (7.7)
Malignancy[b]	10 (9.4)	5 (6.5)	7 (10.1)	3 (8.3)	1 (7.7)
Metabolic[c]	6 (5.7)	5 (6.5)	5 (7.2)	1 (2.8)	0
Hepatitis B	4 (3.8)	1 (1.3)	2 (2.9)	1 (2.8)	2 (15.4)
Hepatitis C	2 (1.9)	1 (1.3)	1 (1.4)	1 (2.8)	0
Other[d]	12 (11.3)	16 (21.1)	13 (18.8)	7 (19.4)	3 (23.05)
BMI (kg/m^2)					
≤24.9	41 (38.7)	34 (44.7)	33 (47.8)***	-	1 (7.7)
25.0-29.9	37 (34.9)	22 (28.9)	24 (34.8)	24 (66.7)	6 (46.15)
≥30	28 (26.4)	20 (26.3)	12 (17.4)	12 (33.3)	6 (46.15)
Diabetes mellitus					
Yes	19 (17.9)	15 (19.7)	11 (15.9)	7 (19.4)	1 (7.7)
No	87 (82.1)	61 (80.3)	58 (84.1)	29(80.6)	12 (92.3)
MELD score					
<15	40 (37.7)	26 (34.2)	22 (31.9)	9 (25)	6 (46.2)
≥15	66 (62.3)	50 (65.8)	47 (68.1)	27 (75)	7 (53.8)

AILD = autoimmune liver disease, ASH/NASH = alcoholic/ non-alcoholic steatohepatitis, BMI = body mass index, MELD = model for end-stage liver disease

Categorical data presented as n (%)

a = Include primary sclerosing cholangitis, primary biliary cirrhosis and autoimmune hepatitis

b = Include hepatocellular carcinoma, cholangiocarcinoma and neuroendocrine tumour

c = Include haemochromatosis, oxalosis and α-1 anti-trypsin deficiency

d = Include hepatic venous outflow obstruction, cryptogenic cirrhosis, sarcoidosis, polycystic liver disease and portal vein thrombosis

Significantly different at the level of: *p < 0.05, **p = 0.01, ***p < 0.01, ****p < 0.0005

Table 2.2 Muscular characteristics of LT recipients

	All patients (n = 106)	**Myosteatosis (n = 76)**	**Sarcopenia (n = 69)**	**Sarcopenic obesity (n = 36)**
Myosteatosis	76 (72)	-	52 (75)	25 (69)
Sarcopenia	69 (65)	52 (68)	-	-
Sarcopenic obesity	36 (34)	25 (33)	36 (52)	-
L3 SMI (cm^2/m^2)	42 ± 9	40 ± 8	38 ± 7	40 ± 6
Muscle attenuation (HU)	32 ± 8	28 ± 6	31 ± 9	29 ± 8

L3 SMI = skeletal muscle index at the level of the third lumbar vertebra, HU = Hounsfield units

Categorical data presented as n (%). Continuous data presented as mean ± standard deviation

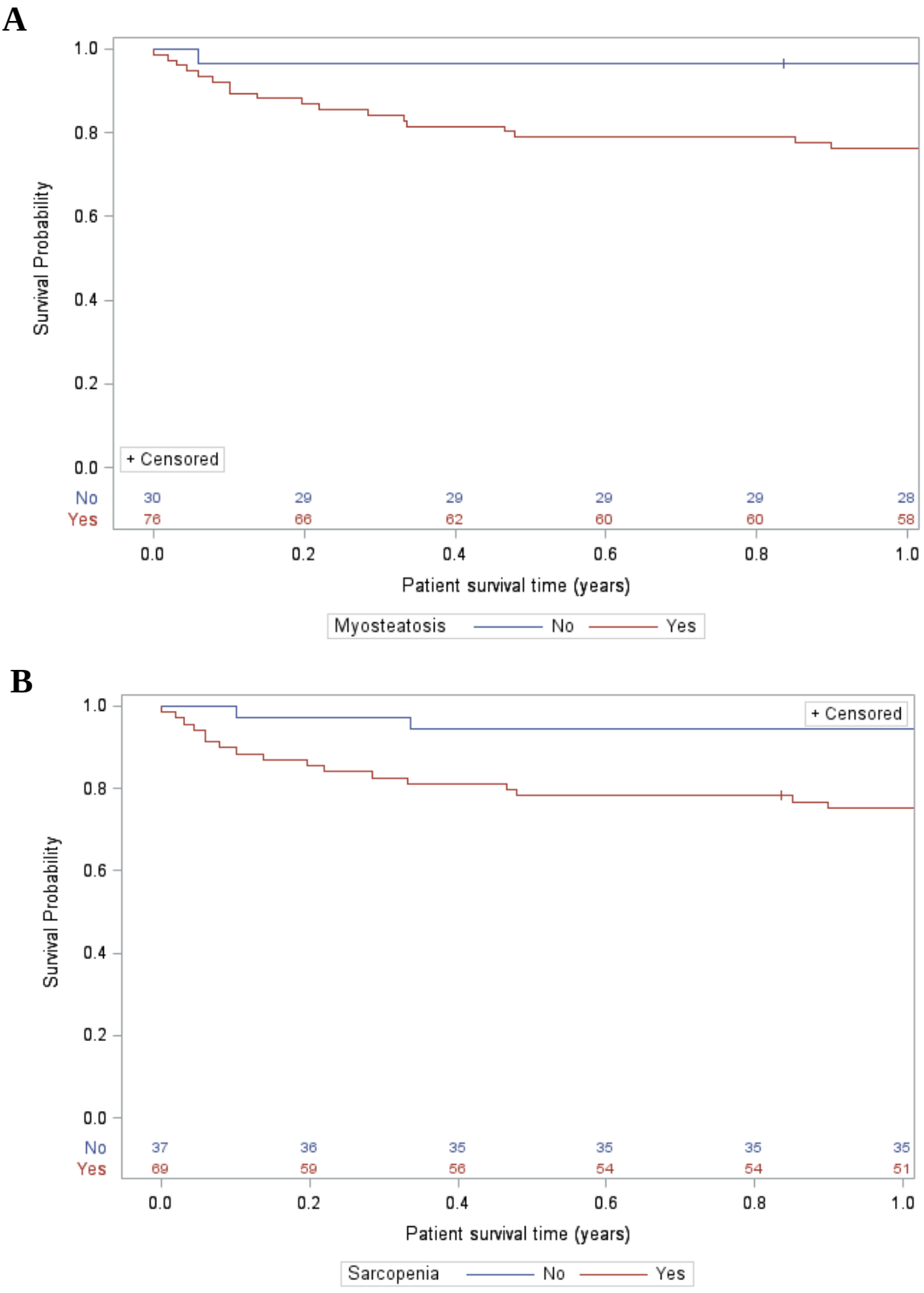

Figure 2.1 Recipient survival in patients with myosteatosis (A) and sarcopenia (B). Survival at one year was significantly lower in patients with myosteatosis than those without myosteatosis (p = 0.049). Patients with sarcopenia had borderline significantly worse survival than patients without sarcopenia (p = 0.051).

Discussion

Patients requiring LT are often severely ill and the elucidation of objective criteria which can assist in optimizing utilization of this scarce resource may assist clinicians. Sarcopenia is a well-studied risk factor for poor outcomes after LT, however, less is known on the prevalence and consequences of myosteatosis and sarcopenic obesity in the transplant setting. To our knowledge, this is the first study describing these muscle abnormalities and their associations in a South African LT recipient population.

In this present study, muscle abnormalities were frequent with myosteatosis seen in over 70%, sarcopenia in 65% and sarcopenic obesity in 34% of the patients. Myosteatosis is emerging as a significant, and possibly better correlate of outcome than sarcopenia in LT recipients. In many studies, and this study, its incidence is higher than that of sarcopenia.[12,17] There were proportionately more females with myosteatosis in our group, an association that has been previously described.[17] The presence of myosteatosis appears to increase length of hospital stay, but this requires further study as the published literature on this is contradictory. Some authors report a significant correlation with prolonged and consequent increased cost of stay, whereas others report none.[12, 15] This study observed that patients with myosteatosis spent on average five days longer in hospital than those without myosteatosis. Similar associations between sarcopenia and hospital stay were not observed.

This study describes, using univariate modelling only, that both low quantity muscle mass (sarcopenia) and low quality fat-infiltrated muscle (myosteatosis) were determinants of mortality in patients with end-stage liver disease one year after LT. The risk of death was

2.5 to three times higher in those with sarcopenia or myosteatosis compared to those without muscle abnormalities. Importantly the prognostic value of myosteatosis was better than that of sarcopenia in predicting patient survival as well as length of hospital stay and allograft survival. In this study none of the three muscle measures were predictive of complications and sarcopenic obesity did not significantly increase the risk for poor survival outcomes.

Although by univariate analysis only, the findings of this study agree with prior publications identifying myosteatosis as a risk factor for allograft dysfunction and mortality.[12, 19] Notably, in this study myosteatosis was a more significant risk factor for mortality than sarcopenia. These results are consistent with those of Czigany *et al*. who measured myosteatosis by the same technique and describe its superiority over sarcopenia in predicting poor peri-operative outcomes including mortality.[15] It is postulated that intramuscular fat accumulation disrupts the architecture and alignment of muscle fibrils weakening their mechanical action.[20] Additionally, immunity is impaired and oxidative stress worsened by local pro-inflammatory cytokines and adipokines which in cohort all seem to predispose to increased complications and mortality.[21, 22] The reasons why myosteatosis might predispose to a worse outcome over sarcopenia are unstudied.

There are limitations to this study. Although many more patients received LT at WDGMC, only a small subset had suitable CT imaging available for review. It is possible that only those with an adverse clinical course were referred for imaging, leading to a selection bias for only the most ill patients. The consequent small sample size, from a single centre, may not represent the entire LT recipient population. The prevalence of body composition

abnormalities may be lower in a more representative sample. Furthermore, there is growing recognition that muscle wasting is one part of a multidimensional frailty syndrome, requiring functional assessment of physical performance and muscle strength as well as measures of muscle quantity or quality.[23, 24] By the retrospective nature of this study, data on these measures such as gait speed or grip strength could not be collected. Lastly, the conclusions are based on univariate analysis only.

This study however serves as a primary analysis of muscle abnormalities in a diverse South African LT recipient population. The clinical relevance of identifying both myosteatosis and sarcopenia in pre-LT assessment is highlighted. As screening tools, they may prove useful predictors of post-transplantation outcome. Nutritional, pharmacological and exercise interventions such as branch chain amino acid supplementation, testosterone supplementation, minimization of immunosuppression and physical resistance training aim to prevent fatty infiltration and wasting of muscle and so may delay functional decline and mortality in these patients.[25-27]

Conclusion

These results suggest that both sarcopenia and myosteatosis infer disproportionately higher risk for mortality at one year after LT, however myosteatosis appears to be a more significant predictor of patient and allograft survival and length of hospital stay. Further studies are needed to determine the underlying pathological mechanisms accountable for this observation.

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SUPPLEMENTARY DATA

Table 2.3 Pre-transplantation variables and one-year survival in liver transplantation recipients

	HR for death at 1 year	**95% CI**	**p-value**
Age (years)			
35-49	0.40	0.12-1.30	0.13
50-64	0.63	0.24-1.69	0.36
≥65	0.67	0.17-2.70	0.58
Sex			
Female	1.30	0.60-2.82	0.50
Self-reported race			
Black-African	2.22	0.93-5.29	0.072
Indian/Asian/Mixed	1.70	0.56-5.19	0.35
Indication for transplant			
ASH/ NASH	0.49	0.20-1.21	0.12
BMI (kg/m^2)			
25.0-29.9	1.15	0.51-2.61	0.74
≥30	0.35	0.10-1.26	0.11
Diabetes mellitus			
Yes	1.69	0.71-4.02	0.24
MELD score			
≥15	1.16	0.52-2.60	0.72

ASH/NASH = alcoholic/ non-alcoholic steatohepatitis, BMI = body mass index, CI = confidence interval, HR = hazard ratio, MELD = model for end-stage liver disease

CHAPTER 3: APPENDICES

Appendix A: United Network for Organ Sharing and Organ Procurement and Transplantation Network model for end-stage liver disease score (59)

Initial MELD score =

$$0.957 \times \log_e(\text{creatinine in mg/dL}) + 0.378 \times \log_e(\text{bilirubin in mg/dL}) + 1.120 \times \log_e(\text{INR}) + 0.643$$

A value of 1.0 is used for any laboratory result less than 1.0.

For candidates with renal dysfunction (creatinine ≥4.0mg/dL) or those who have received two or more dialysis sessions or a single 24 hour session of continuous veno-venous haemodialysis in the past week, a creatinine value of 4.0mg/dL is used in the above formula.

The calculated score is rounded off to the tenth decimal place and then multiplied by 10 to give the initial MELD score. The maximum score is 40.

If the initial MELD score greater than 11, the next formula, accounts for serum sodium level and is used to calculate the final MELD score:

$$\text{MELD} = \text{MELD(initial)} + 1.32 \times (137 - \text{sodium}) - [0.033 \times \text{MELD(initial)} \times (137 - \text{sodium})]$$

A value of 125 is used for any sodium value less than 125 mmol/L and 137 for any sodium result above 137mmol/L.

Appendix B: Child-Turcotte-Pugh score (63)

The original Child-Turcotte score used to grade severity of liver disease was adapted by Pugh to include prothrombin time and exclude body nutritional assessment. Six parameters are assessed to calculate a grade A, B, C, which correlates with operative risk.

Table B1 Child-Turcotte-Pugh score

Clinical and Biochemical Parameters	**Points**		
	1	**2**	**3**
Grade of encephalopathy*	None	1 and 2	3 and 4
Ascites	Absent	Slight	Moderate
Bilirubin (mg/100ml)	1-2	2-3	>3
Albumin (g/100ml)	3.5	2.8-3.5	<2.8
Prothrombin time (seconds prolonged)	1-4	4-6	>6
If primary biliary cirrhosis use bilirubin (mg/100ml)	1-4	4-10	>10

*according to Trey, C., *et al*. (64).

Grade A: 5 or 6 points. Good operative risk.

Grade B: 7-9 points. Moderate operative risk

Grade C: 10-15 points. Poor operative risk

Table B2 Grading of hepatic encephalopathy by Trey, C *et al*. (64)

Grade of encephalopathy	**Mental state**	**Tremor**	**Electroencephalographic changes**
Prodrome (often diagnosed in retrospect) or grade 1	Euphoria, occasionally depression, fluctuant, mild confusion, slowness of mentation and affect, untidy, slurred speech, disorder in sleep rhythm	Slight	Usually absent
Impending coma or grade 2	Accentuation of grade 1, drowsiness, inappropriate behaviour, ability to maintain sphincter control	Present	Abnormal, generalized slowing
Stupor or grade 3	Patient sleeps most of the time, but rousable, speech incoherent, confusion marked	Usually present	Always abnormal
Deep coma or grade 4	Patient may or may not respond to painful stimuli	Absent	Always abnormal

Appendix C: Definitions of abdominal computed tomography scan measurements

Table C1 Definitions of abdominal computed tomography scan measurements

Measurement	Definition	Units	Cut-off value		
Cross-sectional area of muscle (31)	Total cross-sectional area of all the skeletal muscles in a single axial slice image (psoas, erector spinae, quadratus lumborum, transversus abdominus, external and internal oblique, and rectus abdominus muscles) This is measured by outlining the muscles at the standard Hounsfield unit range for skeletal muscle (-29 to +150) using segmentation software.	cm^2			
Skeletal muscle index (31)	Cross-sectional area of muscle normalised by height squared	cm^2/m^2	At L3	Female	<39
				Male	<50
Sarcopenic obesity (16)	Concurrent sarcopenia (skeletal muscle index at L3 meeting above cut-off) AND overweight or obesity (BMI ≥25kg/m^2)				
Intramuscular fat (45)	Mean muscle attenuation in Hounsfield units for the entire cross-sectional area of muscle in a single axial slice. This is measured at the third lumbar vertebra. The value is calculated by the segmentation software programme.	Hounsfield units	BMI <24.9kg/m^2		<41
			BMI ≥25kg/m^2		<33
Cross-sectional area of visceral fat (53)	Total cross-sectional area of the visceral adipose tissue in a single axial slice image. This is measured using segmentation software at the third lumbar vertebra. The cross-sectional areas of tissue deep to the deep fascia and within the standard Hounsfield unit ranges for visceral adipose tissue (−150 to −50) are summed.	cm^2			
Visceral adipose tissue index (53)	Cross-sectional area of visceral fat normalized by height squared	cm^2/m^2	No evidence based pre-existing cut-off value specific to this population is known.		
Bilateral psoas muscle area (38)	Cross-sectional area of the right and left psoas muscles. This is measured by manually outlining the muscle at the standard Hounsfield unit range for skeletal muscle (-29 to +150)	cm^2			
Psoas muscle index (39)	Sum of the area of the right psoas muscle and the left psoas muscle normalized by height squared	cm^2/m^2			
Psoas muscle thickness (36, 38)	Transverse diameter (shortest axis) of the psoas muscle on a single axial slice. This is perpendicular to the axial diameter and manually measured on the right.	mm			
Psoas muscle thickness normalized for height (36)	Transverse psoas muscle thickness normalised by height	mm/m	No evidence based pre-existing cut-off value.		

Appendix D: Data collection sheets

D1. Clinical and biochemical patient variables at the time of liver transplantation

Date of collection:

Date of liver transplantation:

Study participant number:

Record Review Data			
Age (years)			
Sex		Female	Male
Self-reported race			
Height (m)			
Weight (kg)			
BMI (kg/m^2)			
Cause of underlying chronic liver disease			
Serum creatinine (in μmol/L and mg/dL)			
Received two or more dialysis sessions or a single 24 hour session of continuous veno-venous haemodialysis in the past week		Yes	No
Serum Total Bilirubin (in μmol/L and mg/dL)			
INR			
Serum sodium (mmol/L)			
MELD score			
Diabetes mellitus		Yes	No
Donor type		Living Donor	Cadaver
Donor age (years)			
Graft type		Split	Whole
Graft weight (g)			
Duration of cold ischemic time (hours)			
Operative time (hours)			
Duration of hospital stay (days)			
Complications	Surgical re-exploration	Yes	No
	Biliary	Yes	No
	Vascular	Yes	No
	Specify complication		
Patient survival	90 days	Yes	No
	1 year	Yes	No
	Specify days until death		
Graft survival	90 days	Yes	No
	1 year	Yes	No
	Specify days until graft failure		

D2. Computed tomography analysis

Date of collection:

Date of computed tomography scan:

Study participant number:

Total cross-sectional measures			
Parameter to be measured/calculated	**Endplate of L3**	**Endplate of L4**	**Level of the umbilicus**
Cross-sectional area of muscle (cm^2)			
Skeletal muscle index (cm^2/m^2)			
Intramuscular fat (Hounsfield units)			
Cross-sectional area of visceral fat (cm^2)			
Visceral adipose tissue index (cm^2/m^2)			

Psoas muscle measures	
Parameter to be measured/calculated	**Endplate of L3**
Bilateral psoas muscle area (cm^2)	
Psoas muscle index (cm^2/m^2)	
Psoas muscle thickness (mm)	
Psoas muscle thickness normalized for height (mm/m)	

Appendix E: Results and analysis of data not included in the proposed manuscript

Table E1 Relative risk of death at 90 days and one year after liver transplantation

Body composition measure		**n**	**%**	**Death at 90 days**					**Death at one year**				
				No	**Yes**	**RR**	**95% CI**	**p-value**	**No**	**Yes**	**RR**	**95% CI**	**p-value**
Sarcopenic at L3	*Yes*	69	65	58	11	5.90	0.79-43.93	0.08	*52*	*17*	*4.56*	*1,11-18,66*	*0,03*
	No	37	35	36	1				35	2			
Sarcopenic by SMI at L4	Yes	62	58	52	10	3.55	0.82-15.41	0.09	47	15	2.66	0,95-7,48	0,06
	No	44	42	42	2				40	4			
Sarcopenic by SMI at the level of the umbilicus	Yes	59	56	51	8	1.59	0.51-4.97	0.42	47	12	1.37	0,59-3,19	0,47
	No	47	44	43	4				40	7			
Sarcopenic obesity	Yes	36	34	31	5	1.22	0.43-3.45	0.71	29	7	1.13	0.49-2.63	0.77
	No	70	66	62	8				58	12			
Myosteatosis	Yes	76	72	65	11	4.34	0.59-32.18	0.15	*58*	*18*	*7.11*	*0,99-50,89*	*0,05*
	No	30	28	29	1				29	1			
Visceral adipose tissue index (upper tertile ≥41.9cm^2/m^2)	Upper tertile			33	2	0.37	0.09-1.57	0.18	30	5	0.72	0.28-1.85	0.50
	Lower tertiles			60	11				57	14			
Bilateral psoas muscle area L3 (lowest tertile ≤9.94cm^2)	Lowest tertile			29	6	1.74	0.63-4.79	0.28	25	10	*2.25*	*1.00-5.04*	*0.05*
	Upper tertiles			64	7				62	9			
Psoas muscle index L3 (lowest tertile ≤3.68cm^2/m^2)	Lowest tertile			29	6	1.74	0.63-4.79	0.28	25	10	*2.25*	*1.00-5.04*	*0.05*
	Upper tertiles			64	7				62	9			
Psoas muscle thickness normalized for height L3 (lowest tertile ≤10.7mm/m)	Lowest tertile			29	6	1.74	0.63-4.79	0.28	26	9	1.83	0.82-4.08	0.14
	Upper tertiles			64	7				61	10			

CI = confidence interval, L3 or L4 = third or fourth lumbar vertebra, LT = liver transplantation, RR = relative risk, SMI = skeletal muscle index

Significant results are indicted in italics

Table E2 Relative risk of allograft failure at 90 days and one year after liver transplantation

Body composition measure		**Allograft failure at 90 days**					**Allograft failure at one year**				
		No	**Yes**	**RR**	**95% CI**	**p- value**	**No**	**Yes**	**RR**	**95% CI**	**p-value**
Sarcopenic by SMI at L3	Yes	57	12	2.14	0.65-7.12	0.21	51	18	2.41	0.88-6.61	0.09
	No	34	3				33	4			
Sarcopenic by SMI at L4	Yes	51	11	1.95	0.66-5.73	0.22	46	16	1.89	0.80-4.45	0.14
	No	40	4				38	6			
Sarcopenic by SMI at the level of the umbilicus	Yes	51	8	0.91	0.36-2.33	0.84	47	12	0.56	0.45-2.02	0.91
	No	40	7				37	10			
Sarcopenic obesity	Yes	31	5	1.08	0.39-2.99	0.88	28	8	1.11	0.51-2.40	0.79
	No	61	9				56	14			
Myosteatosis	Yes	62	14	5.53	0.76-40.2	0.09	*55*	*21*	*8.29*	*1.17-58.91*	*0.03*
	No	29	1				29	1			
Visceral adipose tissue index (highest tertile ≥41.9cm^2/m^2)	Upper tertile	32	3	0.55	0.16-1.85	0.34	29	6	0.76	0.33-1.77	0.53
	Lower tertiles	60	11				55	16			
Bilateral psoas muscle area L3 (lowest tertile ≤9.94cm^2)	Lowest tertile	29	6	1.52	0.57-4.05	0.40	25	10	1.69	0.81-3.53	0.16
	Upper tertiles	63	8				59	12			
Psoas muscle index L3 (lowest tertile ≤3.68cm^2/m^2)	Lowest tertile	29	6	1.52	0.57-4.05	0.40	25	10	1.69	0.81-3.53	0.16
	Upper tertiles	63	8				59	12			
Psoas muscle thickness normalized for height L3 (lowest tertile ≤10.7mm/m	Lowest tertile	29	6	1.52	0.57-4.05	0.40	25	10	1.69	0.81-3.53	0.16
	Upper tertiles	63	8				59	12			

CI = confidence interval, L3 or L4 = third or fourth lumbar vertebra, LT = liver transplantation, RR = relative risk, SMI = skeletal muscle index

Significant results are indicated in italics

Discussion

Total cross-sectional measures

Sarcopenia measured at three axial levels

In this report, sarcopenia was defined by the SMI at the level of the superior endplate of L3 according to recommended techniques and standardized cut-off values (31, 41). To test the predictive value of SMI at alternative vertebral levels, the measurement was repeated at the superior endplate of L4 and at the level of the umbilicus. The vertebral level of the umbilicus was not consistent amongst participants. It was visualized anywhere between L3 and S3 depending on the presence and degree of ascites and subcutaneous adiposity. In the absence of standardized cut-off values for SMI at these alternative levels, the published cut-off values for SMI at L3 were used. No significant correlations between SMI at L4 or SMI at the level of the umbilicus and patient or graft survival were found. Significant limitations must however be considered when interpreting this data. Most importantly that the cut-off value used is specific to the L3 level and may not accurately reflect sarcopenia at other levels. The population size in this report was too small to accurately determine a cut-off level by optimal stratification. There are no prior studies testing SMI at other vertebral levels. Further research is necessary to determine firstly if musculature at other vertebral levels is reflective of total body musculature and secondly to determine cut-off levels defining sarcopenia.

Visceral fat

In this present report, no significant associations were found between higher visceral adiposity and patient and allograft survival at 90 days or at one year after LT. Interpretation of these results is however also limited by the lack of a standardized gender, sex and

ethnicity-specific cut-off value defining visceral obesity. These findings are in keeping with those of Ebadi *et al*. who measured visceral fat and calculated the visceral adipose tissue index (VATI) by the same technique. In their large study of 677 LT recipients, they determined an optimal cut-off value for VATI predictive of mortality, but report that no statistically significant associations between the two were found (54). Future large studies are necessary to further evaluate the impact of this body composition measure on outcomes after LT.

Psoas muscle measures

Both lower bilateral psoas muscle area and lower psoas muscle index (bilateral psoas muscle area normalized for height squared) measured at L3 were significantly increased the risk of death at one year after LT (RR 2.25, 95% CI 1.00-5.04, p = 0.05). No significant associations were noted between these indices and allograft survival at 90 days or one year and patient survival at 90 days after LT. Published literature on the impact of psoas muscle area measures on mortality is variable. These results contrast findings of Ebadi *et al.* who report that psoas muscle index poorly predicts mortality in recipients on the LT waiting list (39). Multiple studies however report good correlation between low psoas muscle area and mortality (12, 14). Of note again however, interpretation of these indices in the absence of standardized normal values is limited.

Appendix F: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr Natalie Irwin et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180560

NAME: (Principal Investigator)	Dr Natalie Irwin et al
DEPARTMENT:	Internal Medicine Liver Transplantation Centre Charlotte Maxeke Johannesburg Academic Hospital Donald Gordon Medical Centre
PROJECT TITLE:	A retrospective analysis of the impact of sarcopenia on liver transplantation outcomes
DATE CONSIDERED:	25/05/2018
DECISION:	Approved Unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Adam Mahomed
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/08/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **May** and will therefore be due in the month of **May** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

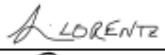
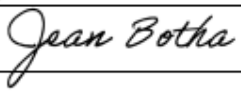
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix G: Declaration of contribution to article and agreement of co-authors

I, Natalie Elizabeth Anne Irwin, 0601818N, declare that this research report is my own work and that I contributed adequately towards research findings in the article stated above.

Signature of Student:  Date: 14/07/2020

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of her research report.

Co-authors	Signature	Date
Dr June Fabian		13 July 2020
Dr Kapila Hari		9 July 2020
Dr Liam Lorentz		10/07/2020
Prof Jean Botha		13/07/2020

Appendix H: Plagiarism declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I _____Natalie Irwin____ (Student number: _0601818N_) am a student registered for the degree of _Master of Medicine (Internal Medicine)_ in the academic year _2020_.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 14/07/2020

Appendix I: Turnitin assessment

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