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Quadriceps Muscle Layer Thickness in Kidney Transplant Recipients: A Potential Measure of Frailty and Sarcopenia

Max A. Levine, *The University of Western Ontario*

Supervisor: Luke, Patrick P., *The University of Western Ontario*
Co-Supervisor: Madill, Janet, *Brescia College*
Joint Supervisor: Sener, Alp, *The University of Western Ontario*

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Abstract

Introduction: Frailty and sarcopenia are related concepts that can impact outcomes after kidney transplantation. Measures of these two entities and new/emerging metrics of sarcopenia remain to be validated.

Methods: In a prospective cohort study, kidney and kidney-pancreas transplant recipients were assessed at the time of transplant with the Physical Frailty Phenotype, bioimpedance analysis, quadriceps muscle layer thickness (QMLT), and CT. The impact on length of stay (LOS), prediction of frailty/sarcopenia, and relative concordance of metrics were analyzed.

Results: Low QMLT, a putative surrogate of sarcopenia/frailty, was more frequently associated with longer LOS (>14 days) after transplant. Additionally, QMLT was predictive of low muscle mass but insufficient at discriminating true sarcopenia, while CT of the abdominal muscles at the L3 level showed good discrimination for sarcopenia.

Conclusions: Further exploration of QMLT and cut-offs for CT and functional metrics in the transplant population are required for future studies and risk stratification.

Keywords

Frailty, Sarcopenia, Kidney Transplant, Bioimpedance Analysis, Physical Frailty Phenotype, Fried Frailty Index, Frailty Phenotype, Kidney Pancreas Transplant, Quadriceps Muscle Layer Thickness

Summary for Lay Audience

Kidney failure leaves patients dependent upon either dialysis or kidney transplant to stay alive. While waiting for transplant, patients can become frail due to age, co-morbid disease, or kidney failure itself. Frailty leads to susceptibility to disease and complications from illness and surgery. Underpinning frailty is sarcopenia, or the loss of muscle characterized by both loss of muscle mass, and strength; having good strength with lower overall muscle mass does not make one sarcopenic.

The relationship between frailty, sarcopenia and the outcomes of kidney transplant, were explored. We studied the utility of using ultrasound measurement of thigh muscles to see if muscle thickness could identify frailty and sarcopenia, and if it could predict how long someone stayed in hospital after transplant. Overall, people with thicker/more muscle had shorter stays in hospital than those with less muscle in the thighs, with an average of 4 days less in hospital (8 vs. 12 days). While significant, those with thicker thigh muscles more often had someone donate a kidney to them, rather than receiving a deceased donor kidney. It is known that living donor recipients have shorter hospital stays, so the impact of thigh muscle thickness is difficult to interpret. Thigh muscle thickness was not clearly associated with frailty, but it did show correlation with low overall muscle mass. Despite this, thigh muscle thickness did not reliably predict sarcopenia, which relies on both muscle mass and function.

Valid measures for identifying sarcopenia in those undergoing transplant are evolving, so we explored how well different measures of sarcopenia compared to CT scan data. We measured the total cross sectional area of the muscles in the torso/core and compared these values to our other measures of muscle (using a body composition analyzer), and strength and walking speed as measures of muscle function. CT provided a relatively good ability to identify sarcopenia (both low muscle mass and strength), with analysis of a single CT picture. This holds promise for refining how we identify patients with sarcopenia in both research and clinical practice with the aim to provide ways of treating/preventing sarcopenia in the future.

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1.1 Brief primer on renal transplantation

End stage renal disease (ESRD) is a chronic health state leaving patients dependent on one of several forms of renal replacement therapy, with initiation of dialysis as the most common and accessible form. The gold standard of renal replacement is kidney transplantation due its impact on longevity, quality of life, and cost-savings.[1-4] As medical care advances, the population of patients on dialysis awaiting a kidney transplant has overall become older, sicker, and made to wait longer for a transplant than initial reports on the benefits of transplant.[3] Despite this, the improved outcomes of transplantation relative to remaining on dialysis are still seen in contemporary studies of transplant outcomes.[3] Recent data suggest that even in the elderly transplant recipients, the overall risk of death may be reduced up to 61% compared to remaining on dialysis, and is associated with an improved life expectancy overall.[5]

The number of patients awaiting a kidney transplant is high, and continues to grow. The Canadian Organ Replacement Register (CORR) reported 3,106 adults in Canada were on the wait list for a kidney transplant in 2017 (with this number increasing year over year), while 38,833 were living with ESRD overall.[6] The rate of kidney transplantation has increased over the last decade, but the number of patients remaining on the wait list remains stable[4]:

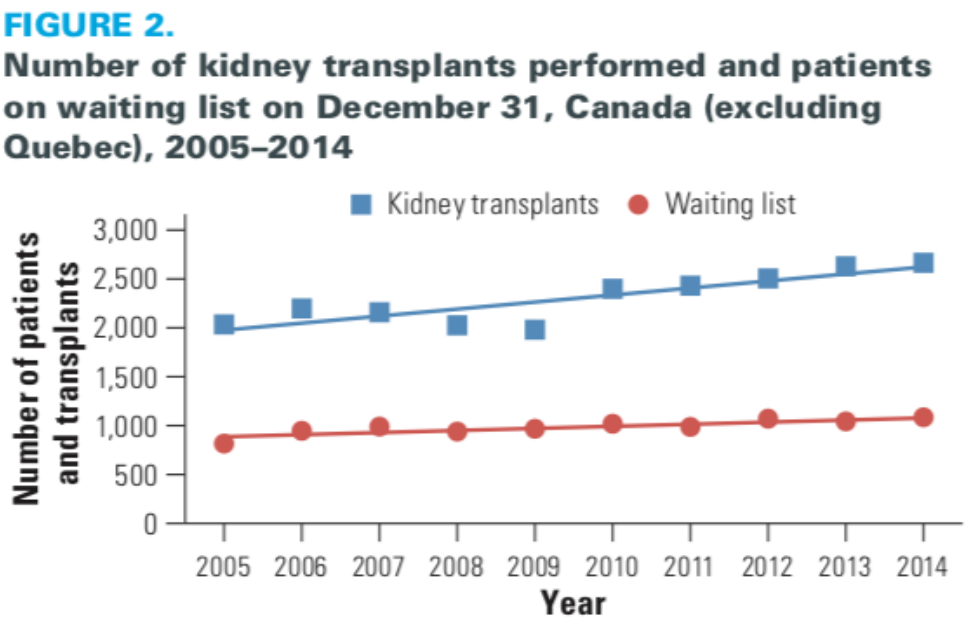


Figure 1. Relationship between the number of kidney transplants performed and the number remaining on the waitlist in Canada

Terner, M., *et al*. (2016). "Increasing Rates of Kidney Failure Care in Canada Strains Demand for Kidney Donors." Healthc Q **19**(3): 10-12.

Given the relative scarcity of access to transplantable kidneys, the priority to optimize outcomes becomes self-evident.

The transplant procedure classically involves an extraperitoneal dissection of the pelvic/lower abdominal vasculature with temporary interruption of blood flow to the lower limb, vascular anastomosis, and establishment of urinary drainage, most often via anastomosis to the native bladder or ureter. Surgery-induced physiologic stress can be significant, with induction of anesthesia, extensive abdominopelvic dissection, and significant fluid shifts. In a recent Canadian report, the rates of Clavien-Dindo Grade 2 or greater complications post-transplant were found to be 60%, with 15% experiencing complications Grade 3b or worse.[7] With the ESRD population waiting longer for transplant, and the average age of this population increasing, careful consideration should be paid to identifying factors that may predispose to poorer outcomes, with the aim of mitigating complications via improved patient selection, risk counselling and stratification, and potentially interventions to alter risk.

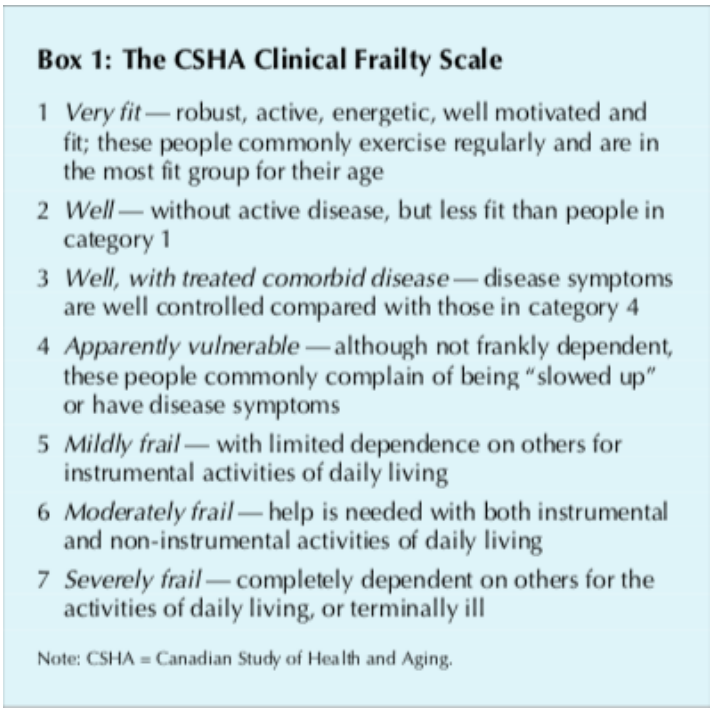
1.2 Review of the Literature

1.2.1 Frailty

The concept of frailty has become an established and refined clinical term, initially arising from the geriatrics literature. Frailty is a complex construct, but has become generally understood and appreciated as a state that represents a loss of physiologic reserve or physiologic dysregulation.[8] In general, the presence of frailty is considered to represent a general inflammatory state that may be associated with a reduced ability to withstand physiologic insults associated with disease.[8-10] Fried and colleagues (2001) contributed significantly to the establishment of a contemporary framework for defining frailty. Frailty had previously been considered similar to being old, disabled, or otherwise lacking potential for longevity, but in recent years has been more accurately defined as a measurable phenotype of an underlying syndrome.[11] This geriatric syndrome has been explored to not only identify patients at risk, but to also differentiate frailty from co-morbidity, as the two concepts are related but not interchangeable.[11,12]

The Frailty Phenotype, commonly referred to as the Fried score, Fried Phenotype, or Physical Frailty Phenotype, defines patients as frail if they possess 3 or more of the following attributes: shrinking (unintended weight loss of ≥ 10 pounds in the prior year), weakness (measured by hand grip strength compared to the weakest 20% of the population), poor endurance or energy (based on self-report of exhaustion), slowness (measured by 15 foot walk test compared to the slowest 20% of the population), low physical activity (a weighted score of energy expenditure per week derived from participant report of activity levels).[11] Those possessing 2 attributes are considered pre-frail, and those with 0-1 are non-frail. Based on data from the Cardiovascular Health Study, a prospective observational study of 5,201 men and women over 65, these five components of the Frailty Phenotype were validated as predictors of falls, worsening mobility, incident hospitalization, and death.[11]

Other means of quantifying frailty exist and are used in clinical practice. Alternative frailty instruments provide clinical practitioners with options that come with their own relative strengths and weaknesses.[12] The Fried score allows for distinct categorization (non-frail, pre-frail, frail) based on an assigned score derived from reported symptoms and performance on tests.[8,11] A competing frailty instrument, developed in a similar vintage, is the Clinical Frailty Scale.[13] The Clinical Frailty Scale (CFS) was derived from data in the Canadian Study of Health and Aging, whereby the initial quantification of frailty began with scoring based on an *a priori* list of clinical deficits (70 in total), and through several iterations of assessment a 7-point scale was created to score frailty based on clinical judgement of a healthcare practitioner.[13]



Box 1: The CSHA Clinical Frailty Scale

1 *Very fit* — robust, active, energetic, well motivated and fit; these people commonly exercise regularly and are in the most fit group for their age

2 *Well* — without active disease, but less fit than people in category 1

3 *Well, with treated comorbid disease* — disease symptoms are well controlled compared with those in category 4

4 *Apparently vulnerable* — although not frankly dependent, these people commonly complain of being "slowed up" or have disease symptoms

5 *Mildly frail* — with limited dependence on others for instrumental activities of daily living

6 *Moderately frail* — help is needed with both instrumental and non-instrumental activities of daily living

7 *Severely frail* — completely dependent on others for the activities of daily living, or terminally ill

Note: CSHA = Canadian Study of Health and Aging.

Figure 2. Clinical Frailty Scale

Rockwood, K., *et al*. (2005). "A global clinical measure of fitness and frailty in elderly people." CMAJ **173**(5): 489-495.

A unifying definition of frailty, and the best way to quantify this concept, remains a work in progress.[12] The relative merits of different approaches to measuring frailty should be considered when applying such instruments to clinical or research scenarios. Overall, the Fried frailty phenotype represents the most often utilized frailty score in the literature.

1.2.1.1 The impact of Frailty on ESRD

While frailty and co-morbidity are distinct concepts, there remains considerable overlap between the "frail" and "co-morbid" populations. One such population of interest include those living with chronic kidney disease (CKD). CKD patients, and especially those with end-stage renal disease (ESRD), are in an extremely vulnerable health state. Given that frailty is often considered an inflammatory state, and ESRD is also associated with chronic inflammation, a shared pathophysiology becomes evident or at least plausible.[10] In a systematic review by Chowdhury and colleagues (2017), the relationship between frailty and CKD was explored.[10] In this review, most studies examining frailty in the setting of CKD have utilized the Fried phenotype, with 27 of 32 studies using it as their determinant of frailty. Despite the apparent consensus on its use, there were several variations of its use, with alternative interpretations of the components of the phenotype. The second most common metric of frailty was the CFS, employed by 3 of the 32 reviewed studies. Other instruments used included the FRAIL scale, Groningen Frailty Indicator, Montensanto approach, Edmonton Frail Scale, and frailty checklist.[10] When the Fried score was used to assess frailty, the prevalence of frailty ranged from 14-73% in dialysis dependent populations. The vast discrepancy between studies is attributed to modifications of the Fried scoring system, where patient reported scores in place of objective measurements are thought to over-estimate the prevalence of frailty.[10]

The relationship between renal dysfunction and frailty has been demonstrated in large prospective studies. Dalrymple *et al* (2013) examined the relationship between degree of renal dysfunction and both prevalent and incident frailty. Drawing from the population in

the Cardiovascular Health Study (community dwelling adults over the age of 65, without evidence of Alzheimer disease, Parkinson disease, stroke), 4,150 individuals were stratified by renal function to define the population exposure of interest, and the outcome of frailty was assessed at two time points. Renal function ($eGFR_{cys}$) was quantified by the 2008 CKD-EPI cystatin-c equation, and frailty by the original Fried phenotype score. Prevalent frailty was quantified at a specific time point in the longitudinal study, and incident frailty derived 4 years after. The relationship between renal function and prevalent frailty was strong, with decreasing $eGFR_{cys}$ associated with higher rates of frailty[14]:

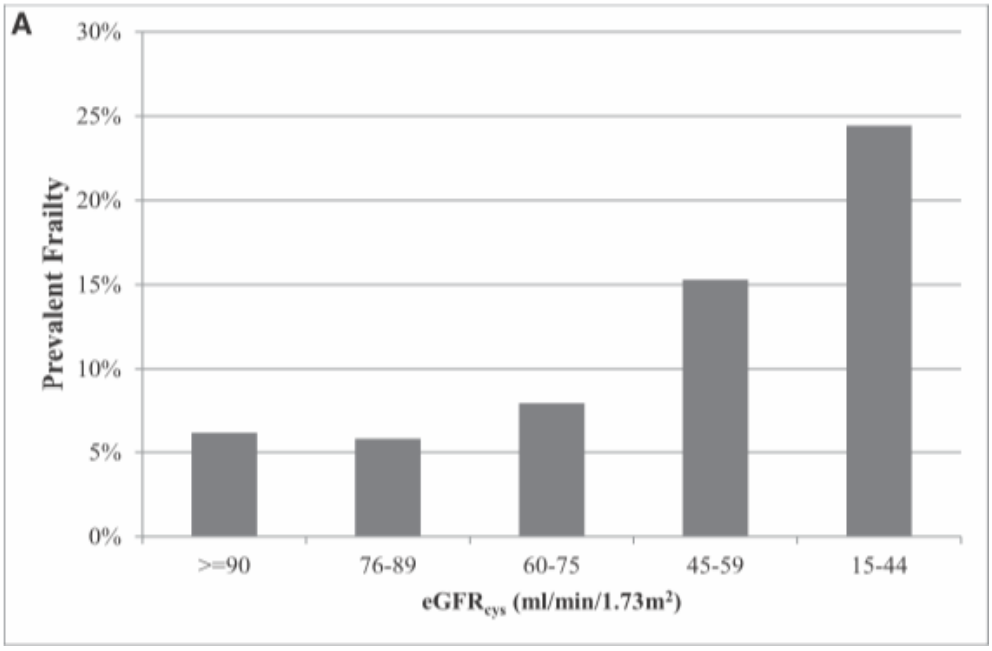


Figure 3. The prevalence of frailty stratified by level of kidney function

Dalrymple, L. S., *et al*. (2013). "Kidney function and prevalent and incident frailty." Clin J Am Soc Nephrol **8**(12): 2091-2099.

Incident frailty followed a similar trend. Logistic regression analysis estimated an adjusted incidence rate ratio for frailty of 3.08 in those with $eGFR_{cys}$ 15-44 mL/min/1.73m^2 compared to those with $eGFR_{cys} \geq 90$ mL/min/1.73m^2. These findings

lend support to a shared pathophysiology between CKD and frailty, since the onset of frailty was not heralded by the initiation of dialysis in this population. Clearly, frailty sets in prior to the need for renal replacement.[14]

The prevalence of frailty at the time of dialysis initiation has also been described. Bao and colleagues (2012) reported on a population undergoing dialysis initiation in an effort to delineate if frailty drove dialysis initiation at higher relative eGFR levels.[15] In this examination of data from the Comprehensive Dialysis Study, prospective assessment of incident maintenance dialysis was related to frailty phenotype at the time of initiation. Frailty was assessed using a modified version of the Fried phenotype that had been published previously, whereby weight loss was omitted due to non-capture of data, slowness and weakness defined by a score of <75 on the physical functioning scale of the SF-12 survey, and exhaustion criterion defined by responses to other items on this survey. The overall prevalence of frailty was 73% in this population, with a prevalence of 63% in patients under 40 years of age. Being frail was significantly associated with a higher mean eGFR at initiation of dialysis (10.4 vs 8.8 mL/min/1.73m^2) as well as a higher risk of death (HR = 1.57 at median 2.9 years of follow up). The higher eGFR at initiation of dialysis may represent the misappropriation of symptoms as uremic complications as opposed to manifestations of frailty.[15] On the other hand, uremia may be contributory to the clinical phenotype that defines frailty according to the Fried score. These authors estimate that the difference in eGFR at the time of dialysis initiation translates to an average of 3 additional dialysis free months in the non-frail.[15]

1.2.1.2 The impact of Frailty on Kidney Transplant Outcomes

The impact of kidney transplantation on outcomes in ESRD is well appreciated, with demonstrable improvements in quantity and quality of life.[1-3] Overall, these benefits of kidney transplant are seen in frail populations as well[9], but frailty may influence the outcomes of those undergoing transplantation. It has been demonstrated in a multi-centre

cohort study of patients on the waitlist for kidney transplant that those deemed frail using the Fried phenotype experience a significantly increased risk of waitlist mortality (HR = 2.19).[16]

The impact of frailty on outcomes post-transplant are manifold. In a prospective study of 183 patients with ESRD at a single institution, Garonzik-Wang and colleagues (2012) demonstrated that frailty predicted the occurrence of delayed graft function (DGF) in kidney transplant recipients.[17] These authors found the prevalence of frailty (defined using the Fried phenotype) to be 25% in their population of patients undergoing kidney transplant. Frailty was associated with a DGF rate of 30% compared to a DGF rate of 15% in the non-frail. After adjustment for donor and recipient confounders, the relative risk of DGF was calculated at 1.94. Interestingly, the effect of frailty was found to be independent of age.[17] This reinforces the idea that frailty is related to but not entirely driven by age. An explanation for the relationship between frailty and DGF is thought to be due to systemic inflammation[17], as frailty represents a manifestation of a pro-inflammatory state and DGF may be related to pre-transplant inflammation.[18] The influential role of frailty on rates of DGF may potentially contribute to risk stratification as well as refine perioperative risk counselling.

Further examination of the potential influence of frailty on kidney transplant outcomes has taken place in the intermediate post-operative period as well. A prospective cohort analysis by McAdams-DeMarco *et al* (2013) found that frailty predicted early re-admission to hospital after kidney transplant. In their cohort of 383 kidney transplant recipients, the authors found that frail recipients, assessed by the Fried phenotype, were more likely to experience early hospital readmission, defined as ≥1 hospitalizations within 30 days of discharge (46 vs 28%). After adjustment for several possible confounders including sex, age, BMI, donor factors, and immunologic risk, frailty conferred an adjusted risk ratio of 1.61 for the occurrence of early readmission.[19] Consistent with other reports, the impact of frailty did not differ with age. When these

authors added frailty to a previously published registry-based model to predict readmission post-transplant, the receiver operator area under the curve was significantly higher with the addition of frailty to the model.[19] Again, frailty represents a measurable factor that can help further risk stratify kidney transplant recipients as well as those who are pending transplant.

Length of stay after transplant has also been shown to be influenced by frailty in a hybrid registry-augmented analysis.[20] Using data (74,859 patients) from the Scientific Registry of Transplant Recipients, risk factors for prolonged length of stay were assessed with precise estimated coefficients, identifying both recipient and donor factors that predicted prolonged stay in hospital. The authors then linked this registry data with their local cohort of 589 transplant recipients with the added variable of frailty, measured by the Fried score, and used the constrained coefficients of the known predictive factors to estimate the independent influence of frailty. Frail kidney transplant recipients had a 1.14-fold longer length of stay on average, and had a 1.6-fold greater likelihood of hospitalization beyond 2 weeks.[20] This novel data analysis model provided granular estimation of risk from confounding factors affecting length of stay while allowing for the emergence of frailty as an independent risk factor for increased length of stay.

The impact of frailty on kidney transplant outcomes goes further, with implications for mortality as well. In a similar hybrid registry-augmented model described above, the same group of authors identified frailty as being an independent predictor of mortality in the kidney transplant recipient population.[21] After adjusting for relevant confounders, transplant recipients deemed frail by the Fried score carried 2.17-fold increased risk of mortality compared to their non-frail counterparts.[21] As has been seen in the general elderly and CKD populations, the impact of frailty on mortality persists after transplant.

An important consideration regarding frailty is the potentially dynamic nature of the phenotype. Chu *et al* (2019) reviewed frailty scores in a cohort of 569 transplant recipients assessed both at the time of transplant evaluation and at the time of transplant. The authors categorized patients' change in frailty status in one of three ways: binary (frail/non-frail), a 3-category state (frail/intermediate/non-frail), or raw frailty score change. At a median of 1.1 years between assessment and transplant, 22% of recipients became more frail, 24% became less frail, and 54% remained stable in their frailty category. Those who became more frail had a greater than 2-fold increased risk of mortality and hospital stay ≥2 weeks after transplant compared to those who remained stable in their frailty state. Additionally, drivers of worsening frailty seemed to relate to age, history of diabetes, and cause of ESRD.[22] While half of patients remained in their frailty category between the time of assessment and transplant, a balanced number worsened or improved. This highlights the potential for frailty to change over time, and suggests serial evaluation may be required if frailty is to be used for risk stratification for transplant. Understanding the pathophysiologic contributors to frailty may allow targeting of modifiable factors underlying patients' frail states, potentially improving longevity and outcomes of transplantation.

1.2.1.3 Biologic Mechanisms of Frailty

The biologic basis for the clinical phenotype of frailty is complex, but is becoming better elucidated over time. Exterkate and colleagues (2016) reviewed the impact of frailty on kidney transplant outcomes, highlighting the evidence for underlying pathophysiologic mechanisms. In their review, the authors note several unifying mechanisms for frailty relating to cellular senescence and a resultant impaired homeostasis, with eventual dysregulation of both energy metabolic systems and the neuromuscular system.[5]

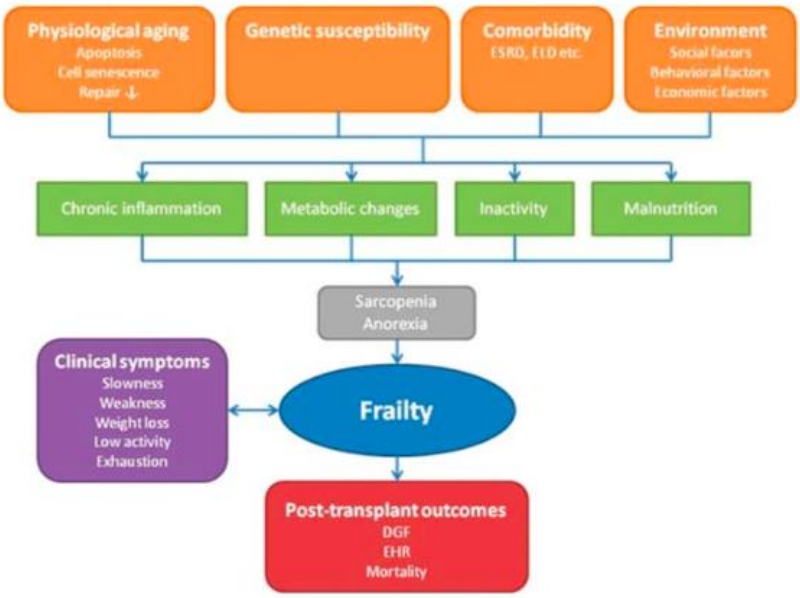


FIGURE 1. Risk factors, pathophysiological changes, clinical symptoms effects on posttransplant outcome. ELD indicates end-stage liver disease.

Figure 4. The relationship of possible risk factors and clinical states contribuitng to frailty in the ESRD population

Exterkate, L., *et al*. (2016). "Frailty and Transplantation." Transplantation **100**(4): 727-733.

Their review describes the immunologic sequelae of frailty, underpinned by evidence of systemic inflammation in the form of heightened levels of Interleukin 6 (IL-6), Interferon gamma (IFN-gamma), and C-reactive protein (CRP). These authors also describe specific immune changes that they consider a hallmark of frailty, including elevated amounts of CD8+ T cells and a lower CD4+:CD8+ ratio, as well as several other alterations in T cell surface markers.[5] Although the clinical significance of these variations in cell surface markers in frail patients is not fully understood now, it stands to reason there may be a potential interplay between frailty and immunologic response to transplant.

1.2.2 Sarcopenia

1.2.2.1 Definitions and Pathophysiology of Sarcopenia

Skeletal muscle comprises a significant proportion of an individual's lean body mass, and accounts for approximately half of the body's total protein energy stores[23]; the loss of lean muscle mass has been termed sarcopenia. As protein is an essential component to the normal function of all organs and organ systems, protein reserves are sometimes called upon to supplement protein and amino acids in states of decreased protein intake or inflammatory disease states.[23] Aging in and of itself represents a significant contributor to sarcopenic changes. "Sarcopenia of aging" is the term granted to age related muscle loss, and begins early in life, with an estimated 0.1-0.5% muscle mass decline per year beginning at age 30, with significant acceleration after age 65.[24] The prevalence of sarcopenia has been estimated at between 5-50% in the elderly general population.[25] No standard or widely accepted definition of sarcopenia existed for several years, but the European Working Group on Sarcopenia in Older People (EWGSOP) in 2010 created a practical clinical definition as follows: "a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life, and death".[24,26] To this end, this group proposed a conceptual staging tool for the presence of sarcopenia including the following criteria: 1) Low muscle mass, 2) Low muscle strength, and 3) Low physical performance.[26] The presence of criteria 1 alone, as measured by some means that accurately estimates muscle mass with comparators to population norms, indicated 'presarcopenia'. 'Sarcopenia' was therefore deemed present when criterion 1 was found in conjunction either criteria 2 or 3, and the presence of all three indicating 'Severe sarcopenia'.[26] Since the proposal of this framework, several other international groups focusing on sarcopenia have provided operational definitions for sarcopenia. There is significant agreement and overlap of these groups' definitions of sarcopenia.[27]

Table 1 Comparison of sarcopenia definitions

Definition	Function	Muscle mass
SIG: cachexia-anorexia in chronic wasting disease [3]	Gait speed <0.8 m/s, OR other physical performance test	Low muscle mass (2SD)
EWGSOP [4]	Gait speed <0.8 m/s; grip strength 40 kg males, 30 kg females	Low muscle mass (not defined)
IWGS Sarcopenia Task Force [5]	Gait speed <1.0 m/s, grip strength	Low appendicular lean mass (<7.23 kg/m^2 in men, 5.67 in women)
Sarcopenia with limited mobility (SCWD) [6]	6 min walk <400 m, OR gait speed <1.0 m/s	Low appendicular lean mass/height^2
Asian Working Group for Sarcopenia [7]	Gait speed <0.8 m/s; grip strength 26 kg males, 18 kg females	Low appendicular lean mass/height^2
Foundation for the National Institutes of Health [8]	Gait speed <0.8 m/s; grip strength 26 kg males, 16 kg females	Appendicular lean mass/BMI

EWGSOP European Working Group of Sarcopenia in Older Persons, *SCWD* Sarcopenia, Cachexia and Wasting Disorders, *IANA* International Association of Nutrition and Aging)

Figure 5. Comparisons of different sarcopenia definitions in the literature

Morley, J. E., *et al*. (2014). "Prevalence, incidence, and clinical impact of sarcopenia: facts, numbers, and epidemiology-update 2014." J Cachexia Sarcopenia Muscle **5**(4): 253-259.

The underlying mechanisms leading to sarcopenia have been described by the EWGSOP and is summarized in the flow diagram below.[26] Sarcopenia represents a manifestation of multiple contributors ranging from lifestyle factors, aging, and sequelae of certain disease states. Furthermore, the EWGSOP suggests sarcopenia can be categorized by etiology, differentiating primary causes (age-related sarcopenia) and secondary causes (sarcopenia of inactivity, disease-associated sarcopenia, nutrition-related sarcopenia).[26]

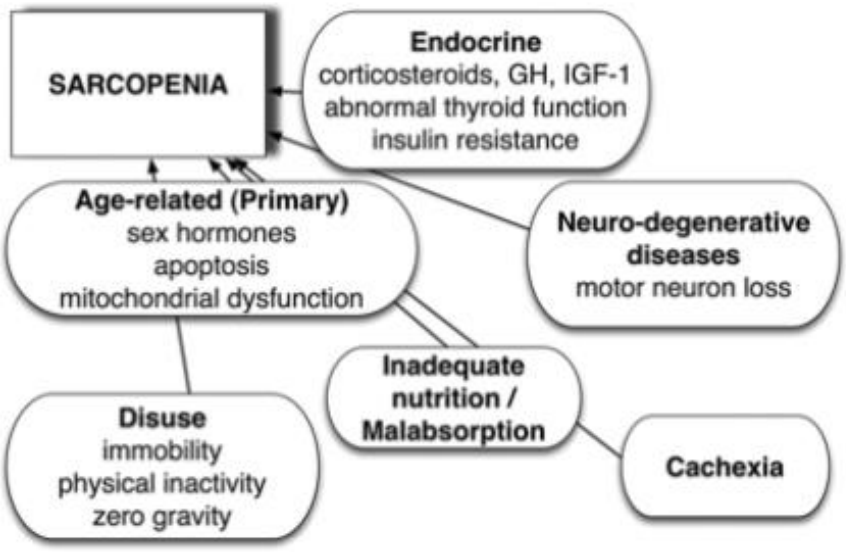


Figure 1. Mechanisms of sarcopenia.

Figure 6. Demonstration of multiple underlying etiologies contributing to sarcopenia

Cruz-Jentoft, A. J., *et al*. (2010). "Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People." Age Ageing **39**(4): 412-423

Further evidence since the initial EWGSOP consensus on sarcopenia has led to an updated framework for the diagnosis of sarcopenia. The EWGSOP2 met in 2018 to update the definition of sarcopenia to be based on the incipient criteria of low muscle strength, with confirmation of the diagnosis heralded by the presence of either low muscle quantity, and/or low physical performance.[28] Muscle strength may be assessed by grip strength or chair stand test. Muscle quantity can be measured using dual energy X-ray absorptiometry (DEXA), whole body or appendicular skeletal muscle mass predicted by bioimpedance analysis, lumbar muscle cross-sectional area by CT or MRI. Physical performance is assessed using gait speed, Short physical performance battery, Timed-up-and-go test, or 400-meter walk test.[28]

In the CKD population specifically, sarcopenic mechanisms have been explored to better understand the pathophysiology of this phenomenon. The metabolic acidosis inherent to CKD disease states leads to activation of the ubiquitin-proteasome degradation pathways, while elevated angiotensin II levels can activate the caspase-3 pathways and causes a reduction in skeletal Insulin-like Growth Factor 1 (IGF-1): these pathways all contribute to muscle protein wasting and apoptosis of skeletal muscle.[29,30] Vitamin D is thought to contribute to skeletal muscle maintenance, and the relative deficiency associated with CKD is considered part of the mechanism for sarcopenia in this population.[30] In fact, supplementation with vitamin D in patients on hemodialysis has been demonstrated to lead to increased thigh muscle cross-sectional area on MRI compared to controls.[31] Additionally, the hypogonadal state of CKD further diminishes skeletal muscle mass through the loss of testosterone's anabolic influence. Testosterone enhances/preserves muscle mass via stimulation of muscle protein synthesis, myoblast differentiation, suppression of myostatin, induction of IGF-1 messenger RNA (mRNA), and supports the regenerative muscle stem cells (satellite cells).[32] In a randomized trial of dialysis patients, testosterone supplementation with or without exercise significantly increased quadriceps muscle cross-sectional area after 12 weeks, whereas control groups did not show any change.[33]

While multifactorial in etiology, chronic systemic inflammation is thought to be a significant contributory factor to the development of sarcopenia, and often stems from chronic illness states, such as CKD.[23,24,34]In a cross-sectional study of patients on hemodialysis for at least 1 year, measurement of muscle mass via CT scanning was undertaken and the values were correlated with serum levels of IL-6 and CRP. Muscle mass, as determined by thigh musculature cross-sectional area, was found to be significantly associated with both inflammatory markers after controlling for dry weight and creatinine kinetics.[34] IL-6 has been reported as a modulator of acute-phase reactant plasma proteins *in vivo* and has been implicated in cancer associated cachexia and muscle breakdown; furthermore, in animal models of IL-6 transgenic mice, antibodies targeting the IL-6 receptor lead to attenuation of proteolysis and muscle atrophy.[34]

In addition, chronic low grade systemic inflammation has been suggested to predispose to sarcopenia via activation of the ubiquitin-protease pathways, diminished anabolic effects of hormones such as IGF-1, and subsequent 'anabolic resistance': a state of relative resistance of muscle anabolism with a given macronutrient intake.[24,29] The heightened levels of oxidative stress associated with aging can induce immune system activation thereby increasing levels of inflammatory cytokines and positively feedback on the deleterious process of reactive oxygen species (ROS) creation in muscle, leading to increased proteolysis, degeneration of neuromuscular junctions, and diminished degrees of excitation-contraction coupling.[24] The sum of these changes leads to loss of muscle mass and impaired muscle function, both considered defining features of sarcopenia.

Oxidative stress is pervasive in states of advanced age, as well as several chronic diseases that often contribute to CKD, in addition to CKD itself. The relative imbalance between ROS and endogenous/exogenous antioxidants leads to a predisposition towards cellular damage resulting in senescent cells.[35] Aging cells are associated with the acquisition of a senescence-associated secretory phenotype that involves the constitutive secretion of chemokines, growth factors, and degradative enzymes.[35] Diabetes mellitus can induce a relative imbalance of ROS via persistent intracellular hyperglycemic conditions, and chronic renal dysfunction can worsen ROS load via several mechanisms including reduced levels of nitric oxide and persistent activation of polymorphonucleocytes.[35] Even in the absence of advanced age, CKD and related conditions (i.e. diabetes) may induce a sarcopenic state through numerous mechanisms.

Evidence for the influence of exogenous inflammation contributing to sarcopenia exists as well. Hemodialysis, known to contribute to systemic inflammation through blood/filtration system interactions, may also contribute to the induction of sarcopenia. Takamoto and colleagues (2018) showed that in a cohort of patients undergoing renal

transplant, the time spent on dialysis was significantly associated with sarcopenia. The degree of sarcopenia was worse when time on dialysis was greater than the median time of the cohort as compared to those with less than the median time on dialysis.[36] The mean±SD age of this cohort was 43±16 years, suggesting the drivers of differences in sarcopenia for this population were likely related to CKD and dialysis itself without a significant influence of age.

The complex nature of sarcopenia was further highlighted in a study of CKD patients where sarcopenia, nutritional analysis, and inflammatory markers were assessed. When compared to non-sarcopenic patients, those who had evidence of sarcopenia did not have significantly different inflammatory marker profiles, nor did nutritional profiles significantly differ.[29] In this cohort, the mean age of sarcopenic and non-sarcopenic patients was 79 and 80 years (respectively), suggesting that the influence of age related sarcopenia and systemic inflammation may affect individuals differently depending on inherent susceptibility.

Because the Fried phenotype of frailty focuses on physical frailty, which stems from muscle function, the physiologic processes behind oxidative stress and subsequent sarcopenia have significant overlap with frailty pathophysiology.[35] Much like frailty's predictive value stems from the ability to quantify and qualify its severity, measurements of sarcopenia require reproducible and objective measures to operationalize its use in patient assessment. The prevalence of frailty is higher in sarcopenic compared to non-sarcopenic patients, even when age, nutritional status, and systemic inflammatory markers were similar.[29] While they are not interchangeable phenomena, frailty and sarcopenia can stem from common pathophysiologic processes, conceptually represented in the flow diagram below.

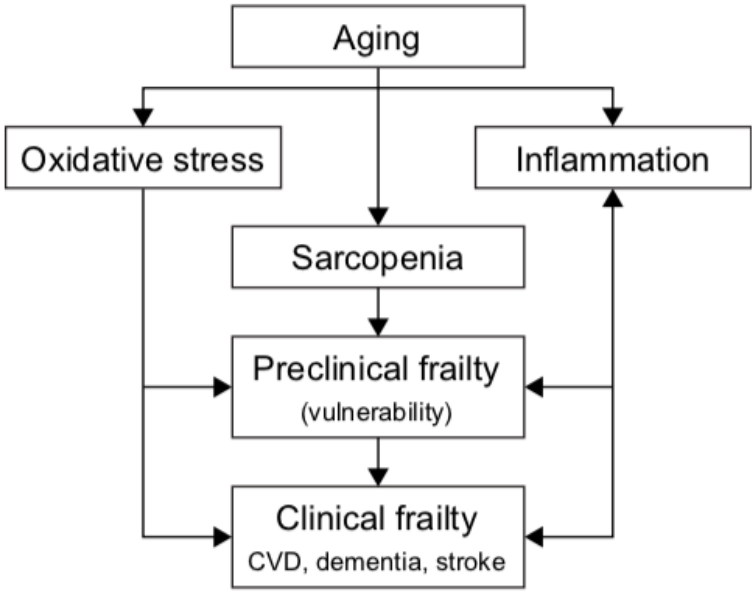


Figure 2 Relationship among oxi-inflamm-aging, preclinical and clinical frailty.
Abbreviation: CVD, cardiovascular disease.

Figure 7. Flow diagram illustrating the possible interplay between aging, sarcopenia and frailty

Liguori, I., *et al*. (2018). "Oxidative stress, aging, and diseases." Clin Interv Aging **13**: 757-772.

1.2.2.2 Measuring Sarcopenia

Functional testing, as the incipient component of sarcopenia assessment, may be done using hand grip strength dynamometry as well as gait speed, among other validated clinical tests.[23] Muscle mass, a component of sarcopenia, may be quantified by one or more other methods. Structural assessment can be obtained via imaging modalities, including cross-sectional imaging using computed tomography (CT) or magnetic resonance imaging (MRI), which allows measurement of specific muscle groups/compartments, as well as dual energy x-ray absorptiometry (DEXA). Cross-sectional imaging has typically been considered the gold standard for assessing muscle mass, but the cost, availability, and potential safety issues with this type of imaging may limit its utility for quantifying sarcopenia.[37] An alternative, lower cost assessment modality includes bioimpedance analysis (BIA). In a study of kidney transplant

recipients, BIA estimations of skeletal muscle mass strongly correlated with psoas muscle volume derived from CT measurements (r=0.761), and was highly correlative with DEXA scanning (r=0.90).[37]

BIA is a method of body composition assessment utilizing bioimpedance, defined as the vector sum of resistance (a measure inversely related to body water content) and reactance of body tissue (a measure of tissue capacitance that varies depending on cell membrane integrity, function, and composition) derived from transmitting low-amplitude imperceptible current passed from the wrist to the ankle.[38-40] Information derived from BIA has been shown to be a reliable metric of body composition in the dialysis population, predictive of clinical frailty, and may be prognostic for survival in hemodialysis patients.[41,42] Data obtained from BIA can provide an estimation of overall health status utilizing the calculation of the bioimpedance phase angle (PhA). PhA is thought to represent the relative health of human tissue at a cellular level, with decreased reactance being reflective of intact, healthy cell membranes.[37] The PhA is derived from calculating the arc-tangent of the reactance divided by the resistance, multiplied by $180°/\pi$.[39] A significant correlation between PhA and survival has been observed in the AIDS, lung cancer and critically ill populations.[39] Furthermore, PhA has been suggested to correlate with survival in those on hemodialysis as well.[43] A visual explanation of the relationship between reactance, resistance and phase angle is shown below.[44]

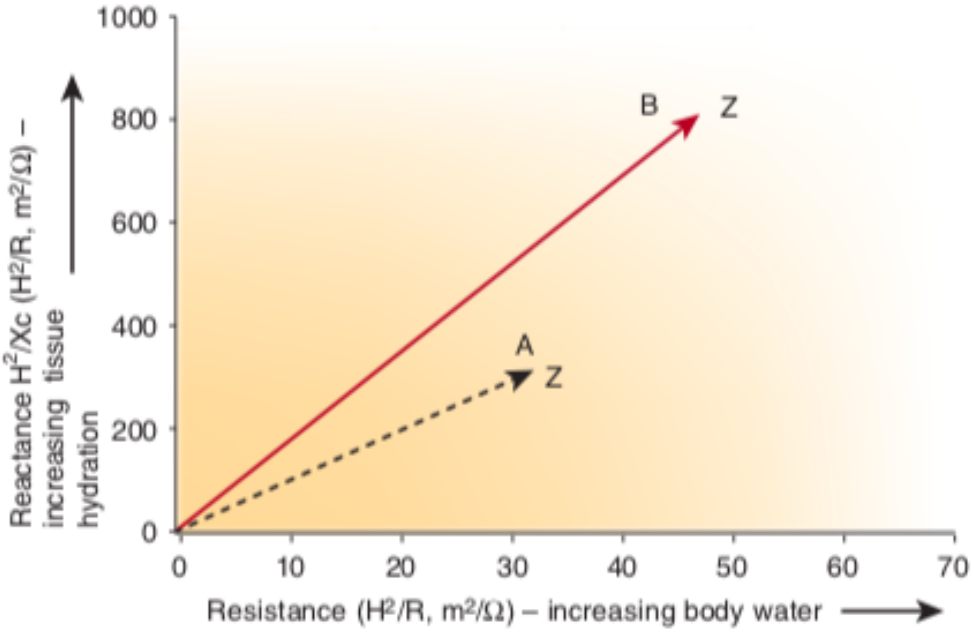


Figure 1 | Impedance to an alternating current can be described as having two components: resistance (*R*) and reactance (*Xc*). Body water is inversely proportional to resistance, and thus H^2/R (X-axis) reflects higher body water content. Cell membranes act as capacitors, and thus increasing H^2/Xc (Y-axis) means less tissue and more extracellular fluid. Patient A is a clinically normovolemic woman; Patient B is a hypertensive hypervolemic man, as shown by his longer and wider vector. In dialysis patients, both the length (*Z*) and angle (θ) of the vector are independent predictors of mortality.

Figure 8. Graph visualizing the relationship between bioimpedance values of resistance, reactance, and phase angle

Davies, S. J. and A. Davenport (2014). "The role of bioimpedance and biomarkers in helping to aid clinical decision-making of volume assessments in dialysis patients." Kidney Int **86**(3): 489-496.

Support for using BIA-derived measures of muscle mass in kidney transplant recipients comes from reports validating this metric. In a cross-sectional study by Ozkayar and colleagues (2014) of 166 kidney transplant recipients, sarcopenia prevalence was assessed using hand grip strength and fat free mass determined by BIA. Overall,

sarcopenia was evident in 20% of the transplant recipient population, with a mean age of 44 in the sarcopenic group and 36 in the non-sarcopenic group.[25] BIA measurement has also been used as a tool to derive absolute values of skeletal muscle mass. Through multiple regression analysis of BIA parameters and patient demographics, Janssen and colleagues (2000) derived an equation to predict whole body skeletal muscle mass as follows:

$$\textit{Skeletal muscle mass (kg)} = [(\textit{Height}^2/R \times 0.401) + (\textit{gender} \times 3.825) + (\textit{age} \times -0.071)] + 5.102$$

where height is measured in centimeters, the gender value is 1 for male and 0 for female, and R is the measured resistance from BIA.[45] This formula was created and validated in a population made of up of Canadian and American volunteers, initially in patients of Caucasian ethnicity and subsequently cross-validated in Hispanic, African-American, and Asian subjects, and showed high correlation with a reference measurement of total skeletal muscle mass derived from whole body MRI:

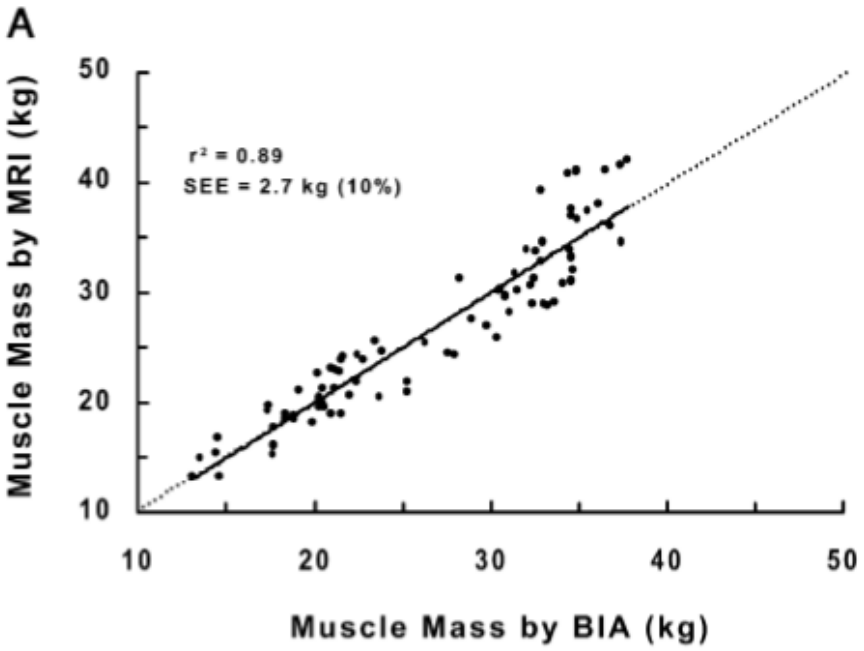


Figure 9. Graph demonstrating the correlation between bioimpedance-derived muscle mass and whole body MRI-derived muscle mass

Janssen, I., *et al*. (2000). "Estimation of skeletal muscle mass by bioelectrical impedance analysis." J Appl Physiol (1985) **89**(2): 465-471.

Healthy volunteers aged 18-86 years old with BMIs ranging from 16-48 kg/m^2 comprised the reference population for this equation.[45] This estimating equation has been applied in other settings to estimate skeletal muscle mass, and has been used in a large population study. The NHANES III study captured BIA measurements in participants, and this data has been used to show that BIA-derived skeletal muscle mass, expressed as a skeletal muscle index (SMI = [muscle mass in kg/total body weight in kg] x 100%), can be used to quantify sarcopenia in the general population and correlates with degree of functional impairment.[46] In this study by Janssen *et al* (2002), NHANES III participants aged 18-39 comprised the referent population, and those in the older age groups (39+) were stratified by SMI, and those between 1 and 2 standard deviations below the referent population were deemed moderately sarcopenic, while those greater than 2 standard deviations below were severely sarcopenic. The results from this report provide population

normative data for a North American population.[46] An alternatively derived SMI, normalizing muscle mass to height squared (kg/m^2), has also been reported in older populations and used to derive cut-off points that increase risk of physical disability.[47] Unfortunately, these reports do not include data on patients with CKD or ESRD/dialysis, and thus these estimating equations have not been validated in the renal failure population.[48] However, population normative data of BIA parameters (tissue resistance, etc.) in the hemodialysis population has been published, therefore derivation of population norms of skeletal muscle mass/SMI could be calculated and explored as a reference for assessment of the dialysis-dependent population.[38]

1.2.2.2.1 Quadriceps Muscle Layer Thickness as a Possible Measure of Sarcopenia

The relative access to body composition metrics, such as DEXA or BIA, may be limited in some clinical settings. Alternative point of care assessments for muscle mass/sarcopenia may be of use to clinicians in patient populations where sarcopenia is prognostic or impact clinical decision making. While whole body assessment of skeletal muscle mass may represent the only true quantification of this measurement, surrogate measurements of regional muscle groups can be representative of whole body muscle mass. In a study of whole body muscle mass with MRI, variations in the relative distribution of muscle were observed and quantified, with pertinent findings displayed below.[49]

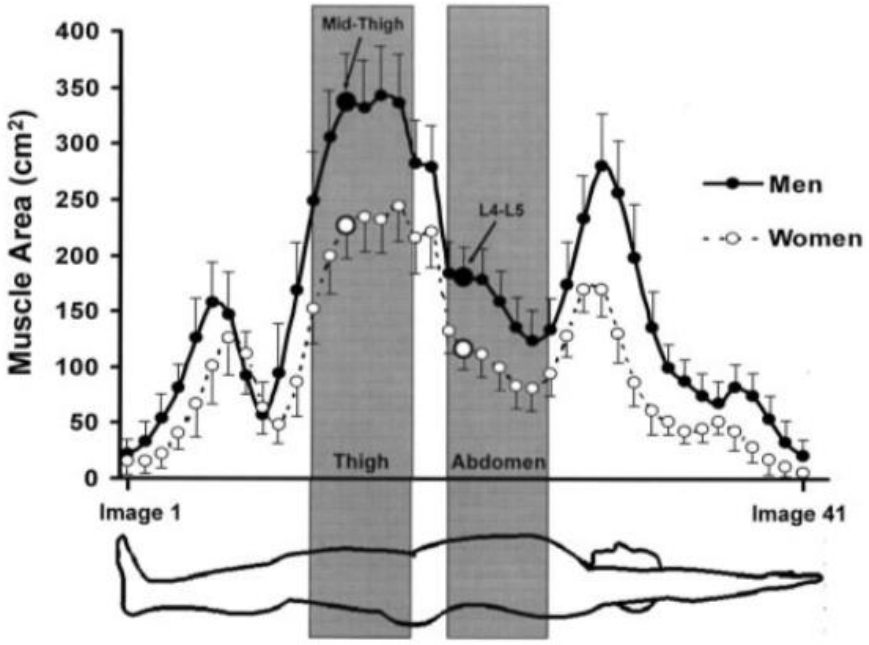


FIGURE 1. Distribution of skeletal muscle as measured by magnetic resonance imaging in 190 men and 197 women. Values are means ± SDs. In general, images 1–16 represent the legs, images 17–19 the pelvic region, images 20–28 the abdomen and torso, and images 30–41 the arms.

Figure 10. Graph showing the relative distribution of MRI-derived muscle mass throughout the body

Lee, S. J., *et al*. (2004). "Relation between whole-body and regional measures of human skeletal muscle." Am J Clin Nutr **80**(5): 1215-1221.

Overall, the cross-sectional area of the thigh musculature represented the highest correlation with whole-body muscle mass with R^2=0.77 and 0.79 for males and females, respectively.[49] Assessment of thigh musculature represents an accessible means for estimation of whole body skeletal muscle mass.

With the relative availability of bedside/point-of-care ultrasound in most clinical settings, application of this technology as an assessment tool for muscle mass has been proposed

in both healthy and ill populations.[50-53] Bedside ultrasound measurement of muscle mass has largely focused on the lower extremity, specifically the anterior thigh compartment.

Anterior thigh muscle can be assessed using point-of-care ultrasound by measuring thickness or cross-sectional area of one or more individual muscles. In healthy older aged females, anterior thigh muscle thickness was higher in those who participated in recreational golfing as compared to sedentary controls.[53] Additionally, the muscle thickness of the anterior thigh compartment of healthy young male volunteers significantly increased after 12 weeks of lower body resistance training, and the increases in muscle thickness were found to correlate with changes in anatomic cross-sectional area as well as MRI-derived volume of the vastus lateralis muscle.[52] These small reports suggest that ultrasound measurement of thigh musculature may be able to capture meaningful variation in muscle mass within and between subjects, creating a rationale for its use as a potential index of sarcopenia and/or frailty.

Assessment of the quadriceps muscle layer thickness (QMLT) has demonstrated predictive value in the general elderly hospitalized population in terms of re-admission, death, and functional decline.[51] In a prospective cohort study of 100 patients aged 65 or greater admitted to hospital for various medical conditions, Guerreiro and colleagues (2017) demonstrated that QMLT was associated with the co-primary endpoint of re-hospitalization or death at 3 months post discharge (RR = 1.24). This relationship was also observed in a bedridden subgroup of the cohort (RR = 1.34). QMLT also correlated with functional testing as well, including gait speed, timed-up-and-go test and handgrip strength.[51]

QMLT measurements have also demonstrated concordance with other measurements of skeletal muscle mass. Berger and colleagues (2015) showed that in healthy community dwelling individuals, QMLT values correlated with lower limb fat free mass (r = 0.74), as

well as total fat free mass derived from DEXA analysis (r = 0.71).[50] Paris *et al* (2017) reported on a prospective multicentre study validating bedside derived QMLT values in critically ill populations. This group found that QMLT values moderately correlated with absolute abdominal muscle cross-sectional area (at the L3 level) derived from CT-scanning, with an r = 0.45.[54] The strength of this relationship was largely driven by the younger male cohort in their population. However, on multivariate regression analysis QMLT alone demonstrated a concordance index of 0.67 in predicting low muscle mass on CT, and this value improved to 0.77 when considered along with other covariates, including age, BMI, gender, type of ICU admission, and co-morbidity index.[54] QMLT may fall short of predicting absolute muscle mass in isolation, but could represent a tool to help stratify patients by category of total CT-derived muscle mass.

The potential application of QMLT measurement to the renal failure population requires consideration of population specific variables, such as the impact of fluid shifts on muscle thickness measurements. Sabatino and colleagues (2017) assessed the reliability of QMLT measurement using bedside ultrasound in the critically ill population with severe acute kidney injury requiring dialysis. In their cross-sectional observational study, when QMLT measurements were compared pre- and post-dialysis, values did not significantly differ. This was regardless of whether conventional 4-hour dialysis or sustained low-efficiency dialysis (6-12 hours) was used, nor did degree of weight change after dialysis impact QMLT measurements.[55] While this population represents acute dialysis as opposed to a chronically dialysis-dependent population, fluid shifts were significant (range of -0.5 to -3.0kg of fluid removed across dialysis sessions)[55], these results provide reassurance that a patient's fluid status likely does not acutely influence QMLT measurements.

Given that bedside ultrasound of the QMLT depends upon a human operator to generate and interpret the images, the validity of this measurement requires documentation prior to its implementation. Reassuringly, the intraobserver variability and interobserver

variability both showed excellent agreement in a cohort study by Guerreiro *et al* (2017)[51], as well as other studies of similar design.[56] Formal study to validate this method of muscle assessment in critically ill populations further supports its validity. Hadda *et al* (2017) sought to quantify the intra- and interobserver reliability of the QMLT assessed by ultrasound in the pneumosepsis population.[57] Measurements were obtained from the right thigh in the supine position with knee extended at the level of the midpoint between the greater trochanter and lateral joint line of the knee in the sagittal plane, with minimal pressure applied to the anterior thigh compartment. QMLT measures involved the muscle tissue just deep to the fascia of the rectus femoris muscle down to the level of just superficial to the periosteum of the femur (includes the vastus intermedius muscle). Two observers independently completed measurements of all participants. The intra-class correlation (ICC) of the intraobserver variability was excellent with values of 0.925 for one examiner and 0.835 for the second; these ICC values suggest almost perfect agreement. Interobserver variability produced an ICC of 0.992, and the mean difference between measurements was -0.082 cm, well within the limits of agreement on Bland-Altman analysis.[57] The reliability of US derived QMLT in the critically ill population has been replicated by other groups. Pardo and colleagues (2018) evaluated QMLT in a population of patients admitted to a surgical critical care unit, obtaining measurements from the two-thirds distance point between the ASIS and upper patella, as well as the mid-point between these landmarks, and quantified changes in QMLT over time in the ICU, as well as intra- and interobserver variability. The ICC of intraobserver variability was 0.74 while the ICC of interobserver variability was 0.76, representing moderate agreement.[58] In contradistinction to the report by Hadda *et al* (2017), these authors performed QMLT measurement with maximal compression of the anterior thigh compartment to compress the muscle to account for potential underestimation of muscle thickness due to tissue edema. The degree of compression was targeted to be maximal pressure without inflicting pain. The degree to which subcutaneous tissue may be compressible likely varies between ages and genders[54] which may have led to variability in compressive force applied within and between assessors. As well, pain infliction may be subjective and vary depending on patients' state of consciousness. Thus, the lower ICC values could stem from this alternative approach to QMLT measurement. On

balance, Paris and colleagues (2016) in a multicentre prospective study of critically ill patients assessed QMLT with the maximal compression method, and returned higher ICC values, with intrarater reliability ICC = 0.98 and interrelater reliability ICC = 0.94.[54] Other reports have demonstrated excellent intra- and interobserver agreement with a minimal compression technique.[50,55,59] With ICC values of reliability ranging from 0.74 to 0.99 among both minimal and maximal thigh compression methods, it appears bedside assessment of QMLT with either technique using ultrasound is likely reliable and reproducible.

Further confidence in the QMLT measurement by bedside ultrasound was presented by Tillquist and colleagues (2014). In their prospective, multicentre international observational study of ICU patients, intra- and interobserver variability between physicians with significant point of care ultrasound experience and trainees that consisted of dieticians, nurses, physiotherapists, coordinators, and technicians were compared; most trainees had no ultrasound experience. Instruction to trainees was provided in the form of a manual, instructional video, and hands-on teaching. Within operator measurements produced an ICC of 0.98 across 12 study locations, and across 78 pairs of trainer-trainee comparisons the ICC was 0.95, with the mean difference in measurements not significantly different.[59] Bedside US derived QMLT measurements across novice and expert examiners create reproducible results, and appears to have validity in the critically ill and dialysis dependent populations.

The application of QMLT to the chronic hemodialysis dependent population (ESRD-HD) has been reported and supports its use as a potential metric of sarcopenia. Sabatino and colleagues (2019) in a cross-sectional prospective observational study evaluated QMLT measurements of HD patients in comparison to healthy and age-matched hospitalized controls. These authors found that QMLT values were significantly lower in the ESRD-HD population.[60] Of note, their QMLT assessment involved minimal pressure application of the ultrasound probe perpendicular to the anterior thigh. Importantly, they

also found measurements performed pre- and post-dialysis were not significantly different regardless of degree of fluid shift, consistent with another report on QMLT in the setting of AKI.[55] QMLT values were moderately correlated with several metrics of protein energy wasting (a contributor to sarcopenia), including BMI ($r = 0.36$) and serum albumin ($r = 0.27$). This report found a stronger correlation between QMLT and the Malnutrition Inflammation Score (MIS), a validated tool to evaluate nutrition and risk of mortality in the renal failure population, where a score of 6 represents the population mean ($r = -0.47$).[60,61] Patients with MIS scores of ≥ 6 had significantly lower QMLT values compared to those having a score of < 6.[60] No reference standard of skeletal muscle mass was available for correlating QMLT (i.e. DEXA scans, CT scans), however there was a clear and consistent relationship between QMLT values and a validated measure of protein energy wasting, which may be considered as a surrogate for a sarcopenic state, and possibly frailty, in the ESRD-HD population. QMLT therefore could represent as a practical tool for bedside estimation of skeletal muscle mass and sarcopenia/frailty, both quantitatively and qualitatively.

1.3 Thesis Objectives

This thesis addresses 3 main objectives by way of an integrated-article format. The overarching theme is the influence of frailty and sarcopenia on kidney transplant outcomes. The interrelated nature of sarcopenia and frailty, as well as the impact both may have on transplant outcomes, makes studying a convenient and clinically accessible surrogate measure attractive. The initial focus of this project was to assess the QMLT, a relatively novel tool, as it relates to frailty and sarcopenia. It was thought that the QMLT could represent a single-step, convenient method for case-detection of frailty/sarcopenia that could improve access to this form of testing in the clinical setting.

First, the QMLT was used as a potential surrogate of frailty/sarcopenia and its impact on clinical outcomes. The relationship between QMLT and length of stay in hospital after

renal transplant was assessed. The QMLT values delineated two cohorts for comparison of length of stay and other related outcomes.

As a follow up, the ability of the QMLT to specifically predict sarcopenia was assessed by defining sarcopenic patients using EWGSOP2 criteria and comparing their attributes to their non-sarcopenic counterparts. This comparison allowed for assessment of the QMLT as a surrogate for identifying sarcopenic patients.

Finally, the agreement and concordance between several different metrics of sarcopenia were examined, including bioimpedance derived-muscle mass, clinical metrics of muscle function, CT measures of muscle mass, as well as QMLT. It was thought these observations will contribute to better appreciating the role each of these modalities might play in assessing sarcopenia in renal transplant recipients.

The current literature is essentially devoid of reports examining those receiving a kidney in concert with a pancreas (simultaneous pancreas-kidney transplant) in the setting of ESRD secondary to type 1 diabetes. There has been suggestion that type 1 diabetes may accelerate loss of muscle mass, and a single report has documented a possibly higher rate of complications in sarcopenic patients undergoing pancreas transplant either with or without a concurrent kidney transpant.[63] We therefore opted to include this population of transplant recipients in this project to add to this nascent area of the literature.

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Chapter 2

2 The Impact of Quadriceps Muscle Layer Thickness on Length of Stay in Those Awaiting Renal Transplant

2.1 Introduction

Frailty has emerged as a relevant prognostic tool in the kidney transplant population.[1] As a construct derived from the geriatrics literature, frailty has come to be recognized as an accumulation of deficits leading to a reduced ability to withstand physiologic stress, in both the general population and those undergoing kidney transplant.[2,3] Those who are frail have been found to have longer length of stay (LOS) in hospital after transplant.[3] Frailty has been most commonly assessed in this population using the Physical Frailty Phenotype, and the scoring reflects measures of muscle function, mass, and activity.[2]

The quadriceps muscle layer thickness (QMLT) represents an emerging form of bedside assessment of muscle mass of the lower thigh, with validation in the critically ill, community dwelling elderly, and healthy populations.[4-7] The QMLT has the potential to serve as a relatively inexpensive screening tool for identifying patients who may be frail.

At present, the QMLT has not been studied in patients undergoing kidney transplant. The relatively low cost and wide availability of point of care ultrasound makes this modality an attractive tool to explore to identify patients at risk or poor outcomes. LOS is a metric of patient outcomes that is associated with reduced global healthcare costs, and is an objective marker of how quickly patients recover after surgery.

2.2 Purpose and Hypothesis

The purpose of this study was to assess whether QMLT values obtained at the time of transplant in those with end stage renal disease are predictive of the total length of stay in hospital following transplant.

It was hypothesized that those patients pending a renal transplant with lower measures of QMLT would have longer lengths of stay following transplant, as well as higher rates of infection and rejection in the early post-transplant period.

2.3 Methods

This is a prospective observational cohort study performed at London Health Sciences Centre University Hospital in Ontario, Canada. Patient recruitment occurred from March 1, 2019 until Jan 1, 2020, where all patients presenting for a kidney or combined kidney-pancreas transplant were invited to participate. Patients were excluded if they were under 18 years of age, receiving a concurrent liver or heart transplant, or refused to participate. Upon recruitment, patients provided written informed consent to participate, at which point data collection occurred which included, age, gender, height, weight, BMI, type of donor (donation after brain death [DBD], donation after circulatory death [DCD], living donor [LD]), panel reactive antibody (PRA%), Physical Frailty Phenotype scores, and quadriceps muscle layer thickness (QMLT).

The Physical Frailty Phenotype was assessed using the criteria set out by Fried *et al* (2001). Components of the Frailty Phenotype included patient reported unintentional weight loss of >10% over the last year, self-reported exhaustion, weekly physical activity, hand grip strength assessed by a Jamar dynamometer, and time to walk 15 feet at one's usual pace. Gender and BMI stratified cut-offs used in clinical practice were

utilized for assessing the presence or absence of each frailty component. A composite score of >2 indicated the presence of frailty. (Appendix 1)

QMLT is a measurement of the anterior thigh compartment musculature comprising the rectus femoris muscle and the vastus intermedius muscle. Point of care ultrasound (BK technology®) was used to quantify this value. With the patient in the supine position and feet pointed forward, a tape measure was used to measure the distance from the anterior superior iliac spine to the superior border of the patella, and the halfway point was marked with indelible ink on the anterior aspect of the thigh in the midline of the lower limb. A curvilinear probe set to 6Hz and with ample ultrasound jelly assessed this point of the thigh musculature. The depth was adjusted so the femur and superficial adipose were visible at the bottom and top of the ultrasound image, respectively. Light pressure was applied to observe tissue dispersion to confirm the discrimination of the visible layers as muscle versus adipose. Pressure was released to the point of minimal pressure from the probe, allowing contact with the probe to the skin with no external compression applied. The image was then frozen, and electronic calipers measured the vertical distance from the inner layer of the rectus femoris muscle fascia to the level of the femur periosteum to obtain the QMLT. This measurement was repeated for a total of three measurements and then repeated on the contralateral thigh. The mean value of the six measurements obtained comprised the patient's QMLT value. An example of the QMLT being measured is depicted in Figure 11.

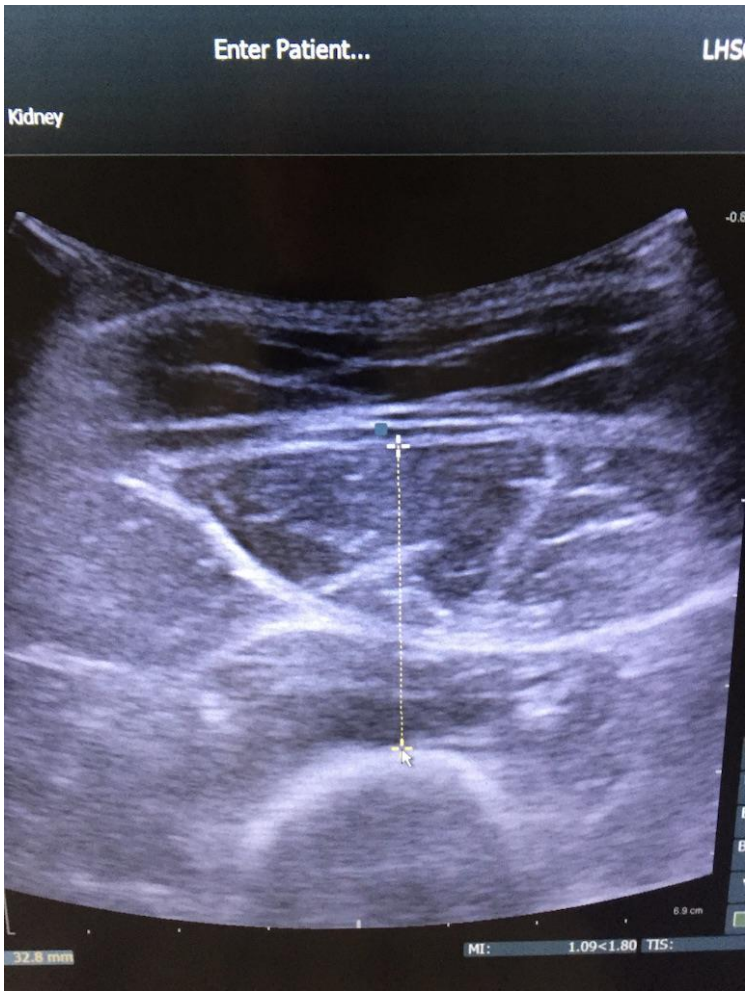

Figure 11. Representative ultrasound image capturing the quadriceps muscle layer thickness and electronic calipers

Patients were then followed prospectively for outcome assessment. The primary outcome of interest was the length of stay in hospital following transplant, assessed both as a continuous variable and as a nominal variable of greater than/equal to or less than 14 days. Secondary outcomes of interest included the occurrence of infectious complication, rejection, and renal function at 1 month post-transplant measured using serum creatinine level; infection and rejection assessment was limited to the first month after transplant. Infection was deemed present if the following criteria were met: culture evidence of

microbial infection with clinical symptoms and/or treatment with antimicrobials, OR radiographic evidence of infection with clinical symptoms and/or treatment with antimicrobials. An infection was not considered present if a patient was treated with a course of antibiotics for prophylaxis or for pre-emptive treatment of a possible donor-derived pathogen. Rejection was documented if there was a biopsy post-transplant documenting graft rejection in the presence of graft dysfunction and treatment provided directed at rejection. Graft function was categorized as delayed if dialysis was required within 7 days of transplant, and was otherwise deemed immediate.

Statistical analysis was carried out using SPSS v 26.0. Demographic data was summarized using descriptive statistics. QMLT values were organized by percentile. A cut-off of the 20^{th} percentile was used to divide patients into two categories of QMLT (low and higher). Kolmogorov-Smirnov testing assessed for assumptions of normality and the continuous outcomes compared using a two-sided t-test or Mann-Whitney U test where appropriate. Chi square analysis and Fisher exact testing for nominal variables were performed. Multivariable regression assessed the predictive impact of QMLT on LOS while controlling for relevant variables. Alpha was set at 0.05 and all analyses were two-tailed. An *a priori* sample size of 74 was chosen to allow for assessing the difference in LOS using unpaired t-test using G*Power 3.1 software, with an effect size of 0.67 based on local data on LOS (mean LOS = 9.0 days, S.D. = 4.5, a 3 day difference in LOS being deemed clinically significant). For the purposes of multivariable regression to be performed on 5 variables of interest, using the guide of 15 patients per variable of interest, a sample size of at least 75 was sought. We therefore aimed to accrue up to 88 patients to allow for a 10-20% rate of attrition. Patients lost to follow up due to early structural graft loss or death were not included in the assessment of our stated outcomes.

2.4 Results

During the study accrual period a total of 85 patients provided consent to participate, with 79 patients providing complete data for analysis. Baseline data is presented in Table 1. The cut-off value to define two QMLT groups at the 20th percentile was determined to be 2.63cm. The range of QMLT was 1.09-6.50cm.

A comparison of the outcome measures based on QMLT group is outlined in Table 2. Kolmogorov-Smirnov testing determined that LOS, PRA%, and creatinine at 1 month violated assumptions of normality and were therefore compared non-parametrically using Mann-Whitney U test. All other continuous variable satisfied assumptions of normality.

The LOS over 14 days was significantly higher in those with lower QMLT. Additionally, LOS was significantly longer in the low QMLT group (12.0 vs 8.0 days, $p = 0.04$). The difference in distribution for the type of donor (living, DCD, DBD) approached significance ($p = 0.052$), in favor of fewer living donors in the low QMLT group. The remaining demographic variables did not differ between the groups, nor did the secondary outcomes of rates of infection, rejection, or creatinine at one month. Notably, the breakdown of frailty phenotype scores did not differ between the groups (Table 2). Given that there was an apparent discrepancy in the rates of living donors between the low and high QMLT groups (7% vs 40%), we repeated the comparisons excluding living donor recipients. The results for this analysis are displayed in Table 3. When LOS was categorized into >14d or ≤14d, the difference was preserved (23% vs 0%, $p = 0.01$). The difference in mean LOS was no longer significant after exclusion of living donor recipients (12.0 vs 9.0 days, $p = 0.22$). This subsequent analysis carried a power calculated *post hoc* of 36%.

Multivariable analysis to assess for contributors of LOS was performed with entry of 5 variables of interest: QMLT, Age, Graft function (delayed vs immediate), Frailty phenotype score, and Donor Type. Because LOS violated the assumptions of normal distribution, LOS was log-transformed for multivariable regression. Overall the model constructed was significantly predictive of LOS, with $R^2 = 0.33$, $F(5,74) = 8.89$, $p<0.001$. The factors of deceased donor and presence of DGF conferred significant regression weight for greater LOS prediction (Table 4).

Table 1. Demographic and Outcome Data for Cohort of ESRD patients Undergoing Kidney Transplant

	Mean	S.D./%
N	79	
Age	49.9	14.4
Gender (%M)	51	65%
BMI	28.7	5.5
QMLT (cm)	3.66	1.14
20%ile QMLT	2.63	
LOS (days)	8.7	4.2
Infection (N)	19	24%
Rejection (N)	5	6%
Frailty score (median)	1	
0	22	28%
1	28	35%
2	12	15%
3	8	10%
4	5	6%
Unknown	4	5%
DGF	17	22%
Donor type		
LD	27	34%
DBD	29	37%
DCD	23	29%
SCD	68	86%

ECD	11	14%
Cr 1 month	138	91
%PRA	25%	38%
Transplant (KTx/SPK)	73/6	92%/8%

* LD = Living Donor; DBD = Donation after Brain Death; DCD = Donation after Circulatory Death; SCD = Standard Criteria Donor; ECD = Extended Criteria Donor; DGF = Delayed Graft Function; KTx = Kidney transplant; SPK = simultaneous pancreas/kidney transplant; PRA = Panel Reactive Antibody; Cr = Creatinine; QMLT = Quadriceps Muscle Layer Thickness

Table 2. Outcome Data of ESRD Kidney Transplant Recipients Between Low and Higher QMLT Cohorts

	<20%ile	>20%ile	p value
N	14	65	
Age (mean)	52.8 (15.3)	49.2 (14.1)	0.4
Gender (%M)	11 (79%)	40 (62%)	0.36
LOS	12.0 (7.4)	8.0 (2.8)	**0.04**
LOS > 14d	3 (21%)	2 (3%)	**0.04**
Infection	2(14%)	17(26%)	0.5
Rejection	2 (14%)	3 (5%)	0.31
DGF	29%	20%	0.65
BMI	28.0 (5.8)	28.9 (5.6)	0.63
LD	1 (7%)	26 (40%)	0.05
DBD	8 (57%)	21 (32%)	
DCD	5 (36%)	18 (28%)	
SCD	11 (79%)	57 (88%)	0.3
ECD	3 (21%)	8 (12%)	
Frailty score			0.7
0	3 (21%)	19 (31%)	
1	6 (43%)	22 (36%)	
2	3 (21%)	9 (15%)	
3	2 (14%)	6 (10%)	
4	0 (0%)	5 (8%)	
Cr 1 months	153 (98)	135 (90)	0.81

PRA	34 (44)	23 (35)	0.18
QMLT	2.07 (0.44)	4.04 (0.90)	
KTx/SPK	12 / 2	61 / 4	

* LD = Living Donor; DBD = Donation after Brain Death; DCD = Donation after Circulatory Death; SCD = Standard Criteria Donor; ECD = Extended Criteria Donor; DGF = Delayed Graft Function; KTx = Kidney transplant; SPK = simultaneous pancreas/kidney transplant; PRA = Panel Reactive Antibody; Cr = Creatinine;

Table 3. Outcome Data of Low and Higher QMLT Cohorts after Censoring of Living Donor Transplant Recipients

	<20%ile	>20%ile	p value
N	13	39	
Age (mean)	54.8 (13.7)	49.9 (15.4)	0.5
Gender (%M)	10 (77%)	22 (56%)	0.324
LOS	12.0 (7.4)	9.0 (2.6)	0.22
LOS > 14d	**3 (23%)**	**0 (0%)**	**0.01**
Infection	2 (15%)	11 (28%)	0.48
Rejection	2(15%)	2 (5%)	0.26
DGF	31%	33%	0.68
BMI	28.3 (4.9)	29.5 (5.3)	0.31
LD	0 (0%)	0 (0%)	0.63
DBD	8 (62%)	21 (54%)	
DCD	5 (39%)	18 (46%)	
SCD	10 (77%)	31 (80%)	1.0
ECD	3 (23%)	8 (21%)	
Frailty score			0.77
0	3 (23%)	11 (30%)	
1	5 (39%)	16 (43%)	
2	3 (23%)	7 (19%)	
3	2 (15%)	2 (5%)	
4	0 (0%)	1 (3%)	
Cr 1 month	155 (102)	146 (112)	0.89

PRA	37% (45%)	29% (38%)	0.4
QMLT	2.05 (0.45)	4.00 (0.92)	

*All data are presented as N(%) or Mean (S.D.)

**DBD = Donation after Brain Death; DCD = Donation after Circulatory Death; SCD = Standard Criteria Donor; ECD = Extended Criteria Donor; DGF = Delayed Graft Function; KTx = Kidney transplant; SPK = simultaneous pancreas/kidney transplant; PRA = Panel Reactive Antibody; Cr = Creatinine;

Table 4. Multivariable Regression Data for Length of Stay (log transformed)

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	1.742	0.219			0	1.305	2.178
QMLT	-0.02	0.035	-0.056	0.95	0.566	-0.09	0.05
Graft function	**0.258**	**0.08**	**0.327**	**1.39**	**0.002**	**0.097**	**0.418**
Age	0.004	0.003	0.142	1.15	0.153	-0.001	0.009
Living vs Deceased	**0.25**	**0.087**	**0.305**	**1.36**	**0.005**	**0.077**	**0.423**
Frail vs Not frail	-0.104	0.09	-0.111	0.89	0.253	-0.283	0.076

*Graft function refers to delayed graft function compared to immediate graft function

2.5 Discussion

In this prospective cohort study of kidney and kidney-pancreas transplant recipients, the QMLT was used as a novel assessment tool for identifying patients at risk for prolonged length of stay in hospital. Overall, those with a low QMLT, defined as measuring below the 20%ile of the cohort, demonstrated a significantly greater proportion with LOS over 14 days (21% vs 3%, p = 0.04), as well as longer mean length of stay in hospital (12.0 vs 8.0 days, p = 0.04).

This difference in LOS may be due to QMLT being representative of frailty. Frailty prevalence was 16% overall (defined as a Frailty Phenotype score > 2). This is lower than other recent reports of frailty incidence in those undergoing kidney transplant, suggesting our population may be more robust than other series of kidney transplant recipients examined for frailty.[8] The components of the Frailty Phenotype include weight loss, slow walking speed, exhaustion, reduced activity, low hand grip strength.[2] Given these measures are derivatives of skeletal muscle use, it stands to reason that frailty may be coexistent with significant loss of skeletal muscle mass and thus could be reflected in the observed quadriceps muscle layer thickness if enough mass was lost overall. In community-dwelling elderly and critically ill populations, QMLT has been shown to moderately correlate with lower limb and total fat-free mass derived from whole body assessments.[6,9]

Frailty has been associated with longer length of stay after transplant. McAdams-DeMarco (2017) utilized SRTR data linked to local institutional data to create a hybrid registry-augmented regression model to precisely estimate the impact of several donor and recipient factors with the ability to estimate the influence of frailty.[3] Through this analysis, frailty conferred a 1.6-fold higher risk of being hospitalized greater than 2

weeks after transplant. We also observed that lower QMLT was associated with a higher risk of LOS > 14 days. We did not observe an association between Frailty Phenotype score and LOS on either pooled analysis or on multivariable regression in our cohort. This may relate to the lower prevalence of frailty in our cohort which may limit the ability to adequately assess this relationship. With a greater number of patients powered to test the relationship between QMLT and Frailty phenotype, a clearer association may emerge.

The rates of the secondary outcomes of infection, rejection, and creatinine at 1 month were not significantly different between low and high QMLT groups. It was hypothesized that QMLT may represent a surrogate marker of frailty, thus representing a state of systemic inflammation and inability to withstand extra stressors.[2,10] Given the known derangements of inflammatory markers such as IL-6 in frail populations, we hypothesized greater rates of infection and rejection.[11] As a result, rates of the clinically relevant insults of infection and rejection were assessed according to QMLT group. QMLT in our cohort was not a robust discriminator of these events. There was a relatively low event rate of rejection overall (6%), thus a larger sample size may help better define the influence of QMLT on rejection. Rates of readmission in larger series, often due to infection or rejection events, after kidney transplant have shown differential rates between the frail and non-frail in other reports.[12] The attendant immunosuppression of all transplant recipients may suppress the influence of frailty on the ability to avoid clinical infection in smaller sample sizes, thus a larger study may be able to better clarify the prognostic ability of QMLT for these events.

A significant driver of increased LOS in our cohort appears to be the type of donor and the presence of DGF. The influence of donor type is first evident by the different rates of donor types between the low and high QMLT groups (Table 2). When LD's were censored, the significance of the difference in mean length of stay was lost (12.0 vs 9.0 days, $p = 0.22$), but the proportion of recipients remaining in hospital beyond 14d

remained significantly higher in the low QMLT group. Eliminating LD's significantly reduced the numbers in each group and this analysis was subsequently under powered to detect a difference in mean LOS. It has been shown in other reports that frailty significantly increases the risk of LOS beyond 14d after kidney transplant.[3] The similar influence of QMLT supports this measure as being potentially indicative of frailty.

The significant impact of donor type on LOS is further clarified by a multivariable regression analysis model using age, graft function, type of donor, frailty status, and QMLT as predictors of LOS. Although both donor type and graft function significantly contributed to regression estimates, this model accounts for only 33% of the residual variation, confirming other factors not included in this model have appreciable influence on LOS. It may be that with greater numbers, QMLT may demonstrate a clearer influence on LOS, and our sample size was not adequate for logistic regression to assess the odds for LOS >14d.

Given that DGF involves dialysis within the first week of transplant, a longer stay in hospital seems natural while waiting to define the progression and trajectory of renal function. DGF has been associated with longer LOS after renal transplant in other series as well.[3] Even in the absence of DGF, deceased donor transplants may display higher rates of "slow graft function", or a less than ideal improvement in renal function without requiring dialysis. This logically leads to longer stays in hospital and has also been demonstrated in larger series.[3]

QMLT is a direct measure of one muscle compartment. Although it was predictive of prolonged LOS, it may not be sensitive enough to adequately reflect clinical frailty. Rather, QMLT may more clearly represent a measure of sarcopenia, or pathologic loss of muscle mass. Sarcopenia is likely a part of the underlying pathophysiology of frailty.[10,11]

The current study was not structured to assess the relationship between QMLT and sarcopenia, but this concept is explored in subsequent chapters.

The discrepant rates of donor types in the low and high QMLT groups may also underscore an unappreciated bias in selecting patients for transplant. It is possible that QMLT may be correlated to some other factor not captured within this study that reduces a patient's likelihood of having or being approved for a living donor transplant. This is another area deserving of further study.

This prospective cohort study represents one of the first to examine QMLT in the renal transplant population, with preliminary suggestion that QMLT may relate to LOS in hospital. There are significant limitations to this study. The current study was powered to detect meaningful differences in LOS and allow for multivariable regression, however the relatively small sample size prevented meaningful subgroup analysis. Further, QMLT provides a metric of muscle size, but it does not measure muscle quality. Fat infiltration of the muscle may be evident subjectively during US assessment of the QMLT by making the muscle appear brighter, but in our current model of QMLT assessment, qualitative assessment of muscle is not incorporated. Future studies investigating the utility of QMLT should consider integrating muscle quality into QMLT assessment. Lastly QMLT measures one compartment of the appendicular musculature, and further research is needed to validate this measurement as a marker of sarcopenia in this population.

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Chapter 3

3 The Relationship Between Quadriceps Muscle Layer Thickness and Sarcopenia in the End Stage Renal Disease Population

3.1 Introduction

Sarcopenia and frailty are two related but distinct conditions affecting the elderly and the renal failure population of all ages.[1] Reports have shown that age alone is not enough to adequately discriminate the presence of these conditions, especially in those with end stage renal disease (ESRD).[2] The importance of sarcopenia and frailty in the ESRD population is gaining appreciation, with longer lengths of stay, higher re-admission rates, and worse mortality after kidney transplantation.[3,4]

The quadriceps muscle layer thickness (QMLT) represents an emerging form of bedside assessment of muscle mass of the lower thigh, with validation in the critically ill, community dwelling elderly, and healthy populations.[5-8] The QMLT has the potential to serve as a relatively inexpensive screening tool for identifying patients at risk of low muscle mass and potentially sarcopenia and frailty.

There is currently a lack of studies examining the utility of QMLT in the ESRD and renal transplant population. Current consensus guidelines recommend sarcopenia be diagnosed

based on functional assessment as well as total muscle mass assessment, the latter often requiring specialized equipment.[9] QMLT has the potential to serve as a tool to detect cases of low muscle mass/frailty in this population.

3.2 Purpose and Hypothesis

The purpose of this study is to assess the relationship between QMLT in the ESRD population presenting for transplant and the presence of sarcopenia and frailty.

It was hypothesized that the QMLT would be associated with measures of sarcopenia as defined by the European Working Group on Sarcopenia in Older Persons (EWGSOP2) criteria.

3.3 Methods

This is a prospective observational cohort study performed at London Health Sciences Centre University Hospital in Ontario, Canada. Patient recruitment occurred from March 1, 2019 until Jan 1, 2020, where all patients presenting for a kidney or combined kidney-pancreas transplant were invited to participate. Patients were excluded if they were under 18 years of age, receiving a concurrent liver or heart transplant, had an implanted defibrillator, or refused to participate. Upon recruitment, patients provided written informed consent to participate, at which point data collection occurred which included, age, gender, BMI, type of donor (donation after brain death [DBD], donation after

circulatory death [DCD], living donor [LD]), physical frailty phenotype scores (which includes hand grip strength and walking speed), quadriceps muscle layer thickness (QMLT), and bioimpedance analysis (BIA).

QMLT is a measurement of the anterior thigh compartment musculature comprising the rectus femoris muscle and the vastus intermedius muscle. Point of care ultrasound (BK technology) was used to quantify this value. With the patient in the supine position and feet pointed forward, a tape measure was used to measure the distance from the anterior superior iliac spine to the superior border of the patella, and the halfway point was marked with indelible ink on the anterior aspect of the thigh in the midline of the lower limb. A curvilinear probe set to 6Hz in B-mode and with ample ultrasound jelly assessed this point of the thigh musculature. The image depth was adjusted so femur and superficial adipose were visible at the bottom and top of the ultrasound image, respectively. Light pressure was applied to observe tissue dispersion to confirm the discrimination of the visible layers as muscle versus adipose. Pressure was released to the point of minimal pressure from the probe, allowing contact with the probe to the skin with no external compression applied. The image was then frozen, and electronic calipers measured the vertical distance from the inner layer of the rectus femoris muscle fascia to the level of the femur periosteum to obtain the QMLT. This measurement was repeated for a total of three measurements and then repeated on the contralateral thigh. The mean value of the six measurements obtained comprised the patient's QMLT value.

Biompedance analysis (BIA) was performed using a Biomarkers 1500 Body Scan Analyzer (©Biodynamics Corporation), utilizing 50kHz current of 1mA. Patients were assessed in the supine position with electrodes place on the dorsum of the right hand and right foot. Output from the BIA included tissue resistance, reactance, and phase angle. The raw values were used to compute total skeletal muscle mass according to the formula by Janssen *et al* (2000), which takes into account patient age (years), height (cm) and gender (male = 1) as well as BIA parameter Resistance (R):

$$\textit{Skeletal muscle mass (kg)} = [(\textit{Height}^2/R \times 0.401) + (\textit{gender} \times 3.825) + (\textit{age} \times -0.071)] + 5.102$$

Total skeletal muscle mass was indexed to the square of the patient's height to derive the skeletal muscle mass index (SMI). An SMI < 10.76kg/m^2 for males and <6.7kg/m^2 for females defined low muscle mass, as per EWGSOP recommended cut-offs.[10]

Hand grip strength was measured using a Jamar dynamometer using the dominant hand in a seated position with the elbow at 90°. Three measurements were taken with the highest value constituting the hand grip strength. Walking speed was assessed by having patients walk 15 feet timed with a stop watch. This was repeated once and the average times were used to calculated the average walking speed (m/s).

Sarcopenia was defined according the EWGSOP2 criteria.[9] The incipient criteria for sarcopenia was hand grip strength below 27kg for males and 16kg for females. If this criterion was met, then the presence of either walking speed <0.8m/s (indicating poor muscle performance), and/or the presence of low SMI (indicating low muscle mass) defined a case of sarcopenia. Patients were then categorized as either sarcopenic or non-sarcopenic. Demographic data and mean QMLT values were recorded. Kolmogorov-Smirnov testing assessed for assumptions of normal distribution of data. Data was compared between the groups using a two-tailed t-test or Mann-Whitney U test for continuous data where appropriate, and Chi-square or Fisher exact testing for categorical data with an alpha of 0.05. A receiver operator curve was constructed to identify the performance of QMLT in predicting the presence of sarcopenia and SMI below gender-stratified cut-offs as binary conditions.

3.4 Results

A total of 79 patients met criteria for inclusion in the study and were analyzed for demographic data and is shown in Table 5. The group was divided by gender to allow for a stratified assessment of sarcopenia. Kolmogorov-Smirnov testing confirmed satisfaction of normal distribution for all continuous variables except for hand grip strength and this variable was thus analyzed non-parametrically.

Other than hand grip strength and skeletal muscle mass index (SMI), the male and female subgroups did not differ in measured variables (Table 6). The incidence of low gender-stratified hand grip strength, the primary criteria for determining sarcopenia, was similar between the males and females (20% vs 21%, p =0.85). Males showed a higher incidence of low SMI compared to females, but a similar proportion scored slow walking speed (<0.8m/s). The incidence of sarcopenia was not significantly different between the groups. QMLT measurements did not differ by gender.

When considering patients with complete data to assess QMLT, demographics and presence of sarcopenia, 74 patients had complete data for analysis (Table 7). The mean age of those with sarcopenia was significantly higher than those without (60.6 [12.5]yr vs 47.8[13.7]yr, respectively, p = 0.01). The mean QMLT value did not significantly differ between those with or without sarcopenia (3.19 [0.90]cm vs 3.74[1.14]cm, respectively, p = 0.17)

The performance of QMLT as a screening tool for sarcopenia, low SMI, and frailty was tested using receiver operator curve analysis (Figures 10, 11). The area under the curve (AUC) for QMLT predicting the presence of sarcopenia was 0.64 with 95% CI of (0.47-0.82), p = 0.17. The AUC for QMLT detecting low SMI was 0.68 with 95% CI of (0.52-0.85), p = 0.04. Regarding frailty prediction, QMLT was not predictive of clinical frailty with an AUC of 0.52 (95%CI 0.35-0.68) p = 0.85; QMLT was not predictive of either low hand grip strength (AUC = 0.60 95%CI [0.44-0.76]) or slow walking speed (AUC = 0.54 95%CI [0.38-0.70]).

Table 5. Demographic and Baseline Data for Entire Cohort

Values expressed as Mean (SD) or N (%)

N	79
Age	49.9 (14.3)
QMLT	3.66 (1.14)
QMLT <20%ile	14 (18%)
BMI	28.7 (5.5)
HGS	33.5 (13.2)
Male	51 (65%)
Sarcopenia	10 (13%)
Frail	17 (22%)
Donor	
LD	27 (34%)
DBD	29 (37%)
DCD	23 (29%)
ECD	11 (14%)
KTx	73 (92%)
SPK	6 (8%)
Slow walk time	18%
Low SMI	24%
SMI kg/m^2	11.2 (2.6)

* LD = Living Donor; DBD = Donation after Brain Death; DCD = Donation after Circulatory Death; SCD = Standard Criteria Donor; ECD = Extended Criteria Donor;

DGF = Delayed Graft Function; KTx = Kidney transplant; SPK = simultaneous pancreas/kidney transplant; Cr = Creatinine; QMLT = Quadriceps Muscle Layer Thickness

Table 6. Comparison of Demographic and Outcome Measures Between Male and Female Recipients

	Male	Female	P - value
N	48	26	
Age	50.0 (14.0)	49.6 (15.3)	0.92
QMLT	3.63 (1.16)	3.71 (1.11)	0.76
QMLT <20%ile	11 (22%)	3 (11%)	0.23
BMI	29.2 (5.6)	27.9 (5.2)	0.28
HGS	38.7 (12.9)	23.9 (6.8)	**<0.001**
Sarcopenia	7 (15%)	3 (12%)	0.72
Frail	6 (12%)	7 (27%)	0.10
Donor			0.74
LD	19 (37%)	8 (29%)	
DBD	18 (35%)	11 (39%)	
DCD	14 (28%)	9 (32%)	
ECD	5 (10%)	6 (21%)	0.14
KTx	48 (94%)	25 (89%)	0.66
SPK	3 (6%)	3 (11%)	
Slow walk time	30 (64%)	12 (57%)	0.60
Low SMI	14 (32%)	1 (5%)	**0.03**

SMI kg/m^2	12.0 (2.19)	9.26 (2.40)	**<0.001**
Low HGS	10 (20%)	6 (21%)	0.85

* LD = Living Donor; DBD = Donation after Brain Death; DCD = Donation after Circulatory Death; SCD = Standard Criteria Donor; ECD = Extended Criteria Donor; DGF = Delayed Graft Function; KTx = Kidney transplant; SPK = simultaneous pancreas/kidney transplant; Cr = Creatinine; QMLT = Quadriceps Muscle Layer Thickness

Table 7. Comparison of Sarcopenic and Non-Sarcopenic Groups

	Sarcopenic	Non-Sarcopenic	P-value
N	10	64	
Age	60.6 (12.5)	47.8 (13.7)	**0.01**
QMLT	3.19 (0.90)	3.74 (1.14)	0.17
BMI	28.5 (5.5)	31.6 (4.9)	0.11
Frail	3 (30%)	13 (20%)	0.44
QMLT<20%ile	3 (30%)	10 (16%)	0.36

QMLT = Quadriceps Muscle Layer Thickness

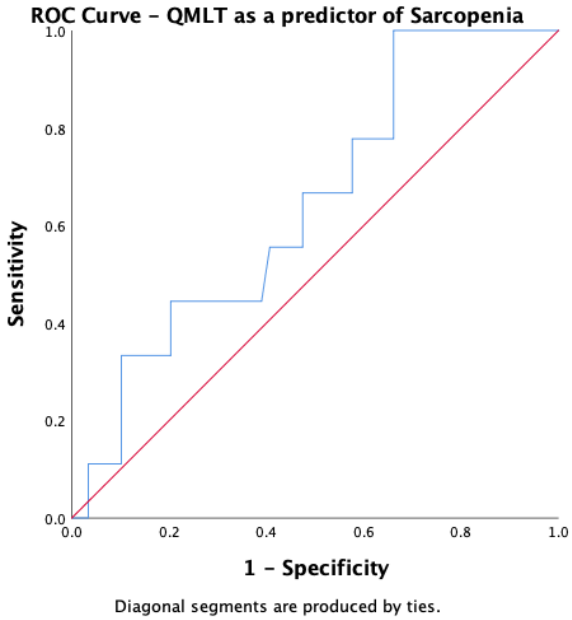

Figure 12. ROC assessing QMLT as a Predictor of Sarcopenia

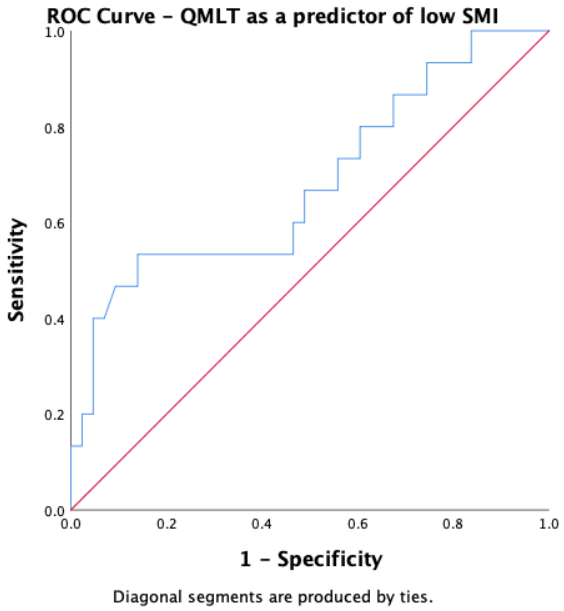

Figure 13. ROC assessing QMLT as a predictor of low Skeletal Muscle Index

3.5 Discussion

In this prospective cohort study of patients with ESRD presenting for kidney or combined kidney-pancreas transplant, the QMLT measurement emerged as a poor predictor for the presence of sarcopenia and frailty. However, the QMLT did demonstrate a significant AUC for the prediction of low SMI as assessed from BIA estimates (AUC = 0.68, p = 0.04).

Sarcopenia was assessed according to the EWGSOP2 consensus on sarcopenia criteria where low hand grip strength (stratified by gender) was the primary indicator of possible sarcopenia, and confirmed with either the presence of low walking speed (<0.8m/s), and/or low muscle mass assessed by whole body skeletal muscle mass index (also gender stratified).[9] The prevalence of sarcopenia was 13% overall, with 12% of males and 15% of females presenting as sarcopenic at the time of transplant. These numbers are in keeping with other reports of sarcopenia prevalence in the ESRD population, with rates of 5.8% to 30.6% cited in the literature, using varying methods of assessment.[1]

QMLT represents an emerging measurement of muscle mass in the ESRD population, and was thought to possibly serve as a source of bedside screening for the diagnosis of sarcopenia. A significant proportion of the body's overall muscle mass can be found in the region of the thighs, justifying the targeting of this region as a surrogate measurement

of total muscle mass.[11] Berger and colleagues (2015) showed that in healthy community dwelling individuals, QMLT values correlate with lower limb fat free mass (r = 0.74), as well as total fat free mass derived from DEXA analysis (r = 0.71).[7] In the critically ill, Paris *et al* (2017) showed in a critically ill population that QMLT demonstrated moderate correlation with the cross-sectional area of the abdominal musculature at the L3 level (r = 0.45).[8] This relationship overall was largely driven by the young males, whereas the correlation in young females, elderly females, and elderly males was not significant (r = 0.13, 0.24, 0.26, respectively).[8] This suggests that QMLT may be applicable as a surrogate measure in select populations. We found reasonable concordance between QMLT and SMI, a measure of whole body muscle mass, but this relationship was not seen regarding sarcopenia.

Several possible reasons may explain the lack of strong association between QMLT and sarcopenia. Firstly, QMLT measures a single compartment of an appendicular muscle group (anterior thigh). While this muscle group is important for locomotion, rising from sitting, and balance, other muscle groups play significant roles in function as well, but are not directly measured by the QMLT. In addition, muscle function defines sarcopenia, with low muscle mass being a potential component.[9] Changes to muscle mass reflected in the QMLT may occur at a later time-point in sarcopenic progression, or distribution of muscle loss may vary based on gender or age. Another consideration is that QMLT measures the thickness of the muscle groups but not the total cross-sectional area of the muscles of interest. Cross-sectional area may provide a better estimation of muscle mass in the lower limb. In the healthy adult population undergoing resistance training, thigh

muscle thickness on ultrasound had moderate correlation with changes in muscle cross-sectional area on MRI (r = 0.69) after training, suggesting the muscle thickness alone is probably a reliable index of muscle cross-sectional area at a given landmark. Thus, measurement of QMLT versus cross-sectional area likely will not significantly impact the relationships observed. Other groups have utilized multiple sites of ultrasound measurement to estimate whole body skeletal muscle, including multiple areas of the thigh and the upper arm, to increase the validity of ultrasound assessments of the appendicular muscles.[8] Lastly, QMLT does not provide a qualitative measure of muscle. Myosteatosis, or fatty infiltration of the muscle, as well as fibrous infiltration of muscle, results from muscle disuse and weakness, but does not necessarily compromise gross approximations of size. Therefore, a muscle with significant myosteatosis may have significantly worse functional potential compared to a similarly sized muscle with minimal steatosis.[22]

Consideration should be given to potential population specific factors, such as fluid status affecting QMLT measurements. Given that impaired management of fluid volume status is a feature of ESRD, it is possible that muscle may swell/contract with hyper/hypovolemic states which could vary from patient to patient depending on adequacy of recent dialysis, dialysis modality, etc. Sabatino *et al* (2017) assessed the reliability of QMLT measurement using bedside ultrasound in the critically ill population with concurrent dialysis dependent severe acute kidney injury. In this cohort, when QMLT measurements were compared pre- and post-dialysis, values did not significantly differ. This observation was preserved regardless of whether conventional 4-hour

dialysis or sustained low-efficiency dialysis (6-12 hours) was used, nor did degree of weight change after dialysis impact QMLT measurements.[12] The fluid shifts experienced in the cohort of Sabatino and colleagues ranged from -0.5 to -3.0kg. In another cohort of ESRD patients on hemodialysis, pre- and post-dialysis QMLT values did not significantly differ. Thus it appears that fluid shifts likely do not impact the assessment of QMLT.

We found in our cohort of kidney and kidney-pancreas transplant recipients a prevalence of sarcopenia of 13% overall (12% in males; 15% in females). This rate is in keeping with or slightly lower than other published reports on sarcopenia in the transplant population. Ozkayar *et al* (2014) showed in a cohort of patients who already received a kidney transplant that sarcopenia was a prevalent in up to 20%, with a mean age of 44 in the sarcopenic group and 36 in the non-sarcopenic recipients. Patients were assessed by hand grip strength using weight based cut-offs from the Cardiovascular Health Study, and BIA derived fat-free mass (not muscle mass per se).[13]

Fluid status is an important factor in BIA assessment. The resistance value provided by a BIA analyzer is a measure that is inversely related to body water content.[14-16] While raw outputs from a BIA analyzer have been shown to be useful in predicting skeletal muscle mass, the concordance of prediction equations to gold standard referent values may be altered by significant fluctuations in total body water.[17] Hydration status impacts the measured resistance, and overhydration may mask muscle atrophy.[1,18] Our protocol did not standardize the timing of BIA analysis in relation to hemodialysis runs or peritoneal

dialysis dwells due to practicalities of data acquisition, potentially reducing the internal validity of this assessment. However, patients were assessed immediately prior to kidney transplant whenever possible. Proceeding to the transplant operation typically requires draining of the peritoneal dialysis fluid and displaying no acute indications for dialysis. Therefore, it is thought the risk of underestimation of muscle mass may be minimal.

Another consideration is interobserver variability of QMLT measurement. QMLT comes from point of care ultrasound measurement, and therefore is susceptible to variability depending on the observer. Hadda *et al* (2017) sought to quantify the intra- and inter-observer reliability of the QMLT assessed by ultrasound in the pneumosepsis population and found that the intraclass coefficient (ICC) of the intraobserver variability ranged between 0.835 and 0.925, with interobserver variability ICC of 0.992.[19] Although we did not specifically test for interrater reliability in our cohort, several reports have demonstrated this modality to be reproducible across both expert and novice assessors.[20,21] Future studies using the QMLT in the kidney transplant population should ensure measures of interrater reliability to add confidence to the internal validity in this population.

The optimal mode of assessment for sarcopenia in the ESRD population remains in evolution. The QMLT may not be a definitive stand-alone tool to replace other validated clinical assessment instruments of sarcopenia, but could play a role in the armamentarium of overall risk stratification of transplant recipients. Further study should be given to the

various forms of identifying sarcopenia to validate the QMLT and other currently available modalities in this population.

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Chapter 4

4 Comparing Metrics of Sarcopenia and Muscle Mass in the Renal Transplant Population – Assessment of Test Performance and Agreement

4.1 Introduction

Sarcopenia, or pathologic loss of muscle, in the end stage renal disease (ESRD) population has been recognized as prevalent and has significant implications on the long term outcomes of those on dialysis as well as those undergoing transplant.[1, 2] Larger muscle mass at the time of transplant has been suggested to portend improved survival after transplant.[1] Although there exists consensus criteria by several international working groups, the most commonly cited diagnostic criteria for the diagnosis of sarcopenias is that of the EWGSOP2.[3] A drawback of the EWGSOP2 criteria is that reviewed studies in the literature have largely excluded the renal failure/dialysis dependent population. In the realm of renal transplant, there is a paucity of data reporting on measures of muscle mass and function to confidently apply widely accepted criteria to this population.

In previous chapters, the EWGSOP2 criteria was applied to a prospective cohort of renal transplant recipients to detect the presence of sarcopenia, however no instruments have been validated specifically in this population. The QMLT has demonstrated association with prolonged length of stay in hospital, as well as shown discrimination for estimates of low overall muscle muscle mass. Some of the metrics used for ascertaining sarcopenia have been applied as part of frailty testing with good validity, namely grip strength and walking speed.[4, 5]

Cross-sectional imaging in the form of whole body CT or MRI has been considered a gold-standard reference for quantifying skeletal muscle content.[3, 6] Whole body MRI or CT is costly, carries extra risk, and is not universally available. Segmental assessment of the body's compartments, such as the mid-thigh, arm, or torso[7] may serve as surrogate measurements that correlate with total body skeletal muscle. However, these forms of imaging have the drawback of being outside of usual clinical practice and thus may suffer from the same drawbacks as whole body imaging.

4.2 Purpose and Hypothesis

The purpose of this chapter is to compare the agreement between different modalities of sarcopenic assessment in the ESRD population undergoing transplant, including muscle mass and functional assessments currently endorsed by the EWGSOP2 criteria as well as the quadriceps muscle layer thickness (QMLT).

It was hypothesized that cross-sectional area (CSA) of the entire abdominal musculature at L3 as well as psoas muscles would correlate with total body skeletal muscle index (SMI) as determined by bioimpedance (BIA) analysis as well as QMLT.

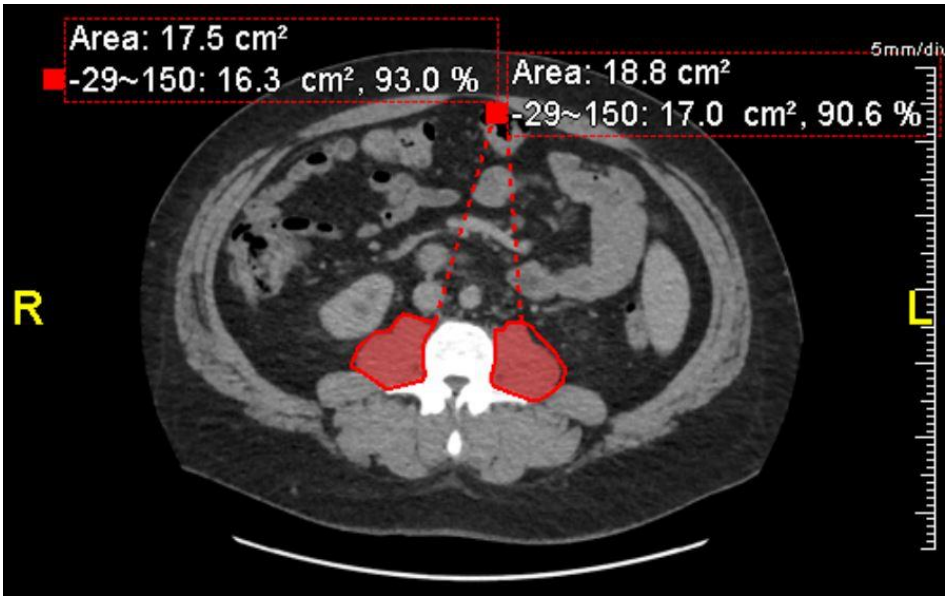
4.3 Methods

This study involved a prospective observational cohort study performed at London Health Sciences Centre University Hospital in Ontario, Canada. Accrual of patients occurred from March 1, 2019 to Jan 1, 2020. All patients presenting for a kidney or combined kidney-pancreas transplant were invited to participate, and were excluded if under 18 years of age, had a pacemaker, were due to receive a concurrent liver or heart transplant, or did not agree to participate. Participants had demographic data collected which included age, gender, height, weight, BMI, type of donor, as well as clinical assessment values related to the physical frailty phenotype described previously[8], in

addition to bioimpedance assessment used to derive whole body skeletal muscle mass.[9] QMLT was measured as well according to the protocol described earlier in Chapter 2 and 3.

When CT scans of the abdomen and pelvis were available for recipients, these images were analyzed for skeletal muscle mass. CT image data sets were imported from AGFA Healthcare PACS (Version 8.1.2) into Aquarius Intuition Viewer (Version 4.4.13.P2 - TerraRecon) to perform body composition analysis. Two consecutive axial image slices at L3 vertebral level were selected for analysis. Skeletal muscle cross-sectional areas (cm^2) for each CT slice were measured by summing the appropriate density pixels in the areas of interest. Two different areas of interest were utilized for analysis. In the first, segmentation of all the skeletal muscles on an axial slice was performed; this included the erector spinae, quadratus lumborum, psoas, internal and external obliques, transverse abdominus, and rectus abdominus muscles. In the second, segmentation of the bilateral psoas muscles alone was performed. Boundaries for the two areas of interest were manually defined for each slice by a subspecialty-trained musculoskeletal radiologist. A CT Hounsfield unit (HU) range of -29 to +150 was used to defined skeletal muscle. Measurements of the cross-sectional area of skeletal muscle for both the "total muscle cross-sectional area" (CSA_{AbdoL3}) and "psoas cross-sectional area" ($CSA_{PsoasL3}$) were performed for each axial slice and then an average of the two slices was obtained for each of the respective cross-sectional areas (Figure 14).

A.


Area: 17.5 cm²
-29~150: 16.3 cm², 93.0 %
Area: 18.8 cm²
-29~150: 17.0 cm², 90.6 %
5mm/div
R
L

B.

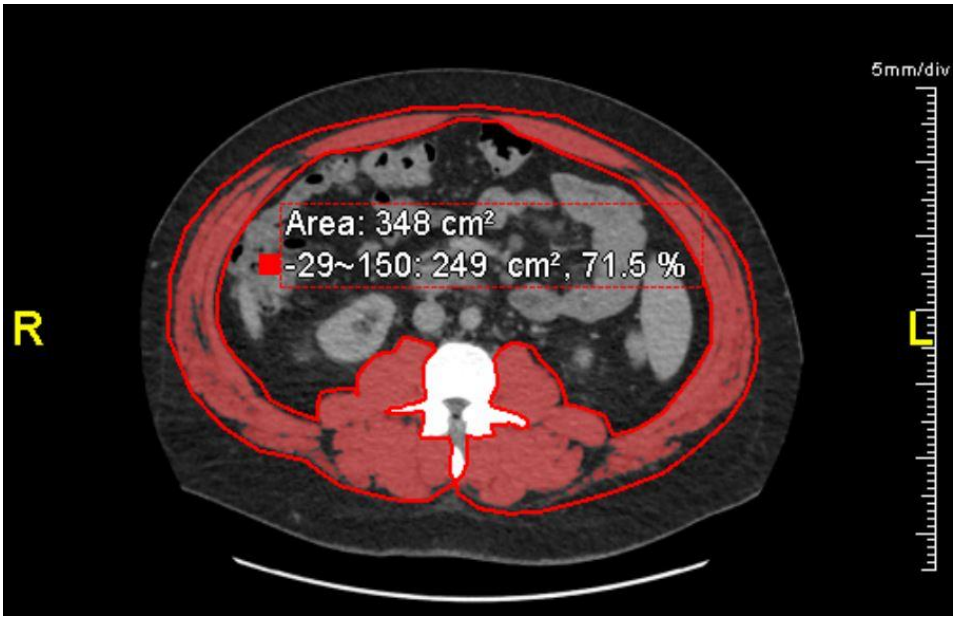


Figure 14. Representative CT image at L3 showing definition of area of interest and area of muscle identified by TerraRecon software based on HU parameters of -29 to 150. A. Psoas cross-sectional area, B. Total abdominal cross-sectional area

CT images were not obtained for the purposes of research. All CTs were done in the context of clinical indication to assess vascular or intraabdominal organ anatomy to aid in surgical preparation for transplant or assess patients for cause. CT images obtained in the peri-transplant period (upon admission, post-operative) were deemed eligible for inclusion in the analysis as it was considered that these perioperative images would still provide reliable representations of the skeletal muscle mass possessed by recipients at the time of transplant. CT images were not included in the analysis if the L3 region was incompletely imaged, edema was too significant to reliably distinguish regions of interest for muscle area determination, or defects in the abdomen were present due to surgical complication (such as CT done for massive dehiscence obscuring the contours and boundaries of the abdominal wall musculature).

Statistical analysis was performed using SPSS v 26.0. Kolmogorov-Smirnov testing assessed for the assumption of normality of the analyzed data. Demographic data was collected relating to age, gender, and presence of sarcopenia. These values were compared between gender groups using unpaired t-test and Fisher exact testing where appropriate. The primary analysis was construction of a correlation matrix of the various measures contributing to the assessment of sarcopenia according to the EWGSOP2 criteria. When variables satisfied assumptions of normality Pearson correlation was used, and when normality assumptions were violated the Spearman correlation was employed. The variables included measures of muscle function (hand grip strength, time to walk 15 feet converted to walking speed), muscle mass (skeletal muscle index [SMI] derived from bioimpedance analysis[9]), $CSA_{PsoasL3}$, CSA_{AbdoL3}, as well as the QMLT. Receiver-Operator Curve (ROC) analysis was performed to assess the performance of the CT derived metrics in predicting the presence of sarcopenia. Sarcopenia was deemed present when low HGS based on gender-stratified cut-offs was identified, and the presence of either slow walking speed (<0.8m/s) or low skeletal muscle mass based on accepted SMI cut-offs.[3, 6] Finally, the agreement between CT metrics ($CSA_{PsoasL3/AbdoL3}$) and the classification of low SMI and sarcopenia was assessed using a Kappa score for each test. Cut-offs of the 25^{th} and 50^{th} percentile for both CT metrics were tested for agreement with the classification of low SMI (based on the threshold for sarcopenia classification), as well as for sarcopenia itself. Alpha was set to 0.05 for all analyses.

4.4 Results

Forty four of 79 patients in our observational cohort had CT scans of the abdomen/pelvis performed prior to, or shortly after their transplant, that were of adequate quality for assessment. Table 8 contains the gender stratified values of CT metrics and rates of sarcopenia. Fewer females were sarcopenic numerically, however this did not meet statistical significance. The median time between CT scan and transplant was 56 days prior to transplant, with 14 of 44 having a CT performed greater than 6 months beforehand (range 187-602 days pre-transplant), while 16 of 44 had a CT performed after the transplant (range 1-130 days post-transplant). The remaining patients' CT scans were performed between 1 and 167 days before surgery (median = 63 days).

Table 8. Gender stratified values of age and CT derived metrics of muscle mass at L3 and sarcopenia

	Male	Female	p-value
N	30	14	
Age (yr)	51.4 (13.6)	50.2 (14.5)	0.79
CSA Abdo L3 (cm^2)	159.9 (28.7)	114.8 (22.6)	<0.001
CSA Psoas L3 (cm^2)	22.6 (5.6)	14.2 (2.7)	<0.001
Sarcopenia (%)	6 (20%)	1 (7%)	0.65

Values for Age and $CSA_{Abdo/PsoasL3}$ are presented as mean (S.D.)

All 30 males had both CT and adequate data to classify sarcopenia, while only 8 of 14 females had complete data. Subsequent analysis was only completed for the males in the cohort due to lack of assessable data in female participants.

Table 9 displays a correlation matrix comparing the univariate correlation of the individual components of sarcopenia classification, the CT derived metrics, and the QMLT. Hand grip strength (HGS) was significantly correlated with gait speed/walk time, $CSA_{PsoasL3}$, and CSA_{AbdoL3}. CSA_{AbdoL3} was most strongly correlated with the $CSA_{PsoasL3}$, and was followed by total body SMI derived from BIA parameters. QMLT showed no significant correlation to any of the sarcopenia/CT parameters.

Table 9. Correlation matrix of sarcopenia parameters, CT measures of muscle mass, and QMLT

	HGS	Walk time	SMI	CSA Psoas L3	CSA Abdo L3	QMLT
HGS	1.0	-	-	-	-	-
Walk Time	-0.34**	1.0	-	-	-	-
SMI	0.25	0.08	1.0	-	-	-
CSA Psoas L3	0.57**	0.02	0.28	1.0	-	-
CSA Abdo L3	0.64**	-0.01	0.51*	0.71*	1.0	-
QMLT	0.08	-0.11	0.19	-0.02	0.14	1.0

* indicates $p<0.05$ for Pearson correlation coefficient

** indicates $p<0.05$ for Spearman correlation coefficient

Receiver-Operator Curve (ROC) analysis assessed the performance of the CT metrics in predicting sarcopenia. Table 10 contains the associated Area Under the Curve (AUC) values for both metrics as well as age, a known contributor to sarcopenia. The CSA_{AbdoL3} demonstrated good performance in predicting the presence of sarcopenia with an AUC of 0.81 (95%CI: 0.66-0.97, p = 0.02). $CSA_{PsoasL3}$ and Age did not significantly predict sarcopenia, although age approached the level of significance. The ROC curves for the performance of CSA_{AbdoL3} is depicted in Figure 15 and 16. The non-significant ROC curves are not displayed. The cut-off point for optimal performance of CSA_{AbdoL3} was $155cm^2$, which was associated with a sensitivity of 83% and a specificity of 74% for predicting sarcopenia. Changing the cut-off to $159cm^2$ (the median measurement) increased sensitivity to 100% while specificity decreased to 65%.

Table 10. Areas under the curve for CT metrics and age for identifying sarcopenia and skeletal muscle index below gender-stratified cut-offs

Variables	AUC	p-value	95% CI
CSA L3 total : Sarcopenia	0.81	0.02	0.66-0.97
CSA L3 psoas : Sarcopenia	0.52	0.87	0.25-0.79
Age : Sarcopenia	0.72	0.06	0.50-0.95
CSA L3 total : Low SMI	0.74	0.03	0.53-0.95
CSA L3 psoas : Low SMI	0.48	0.88	0.26-0.71
Age : Low SMI	0.50	0.97	0.29-0.70

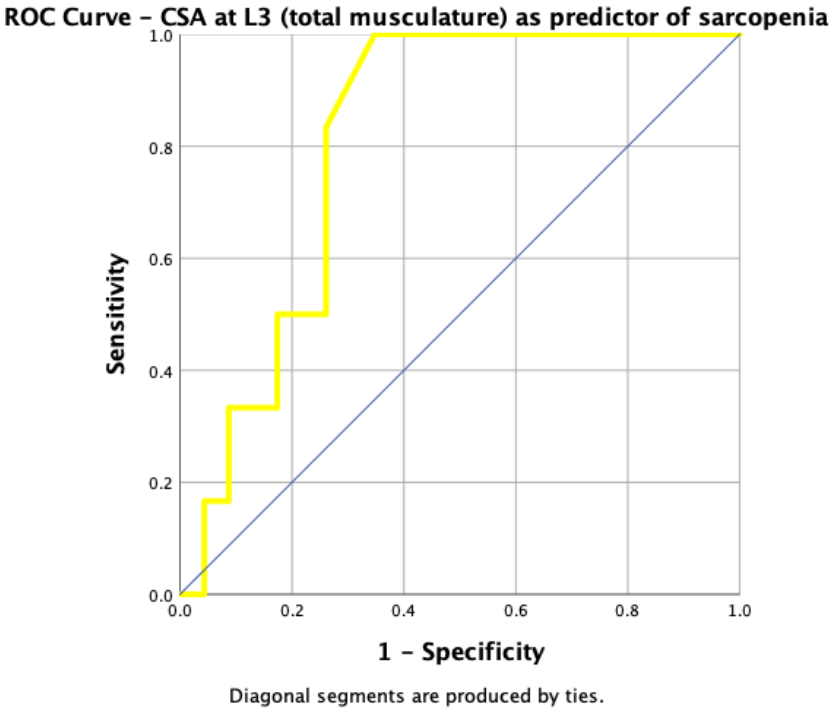


Figure 15. Receiver operator curve of cross-sectional area of total abdominal musculature at L3 as a predictor of sarcopenia

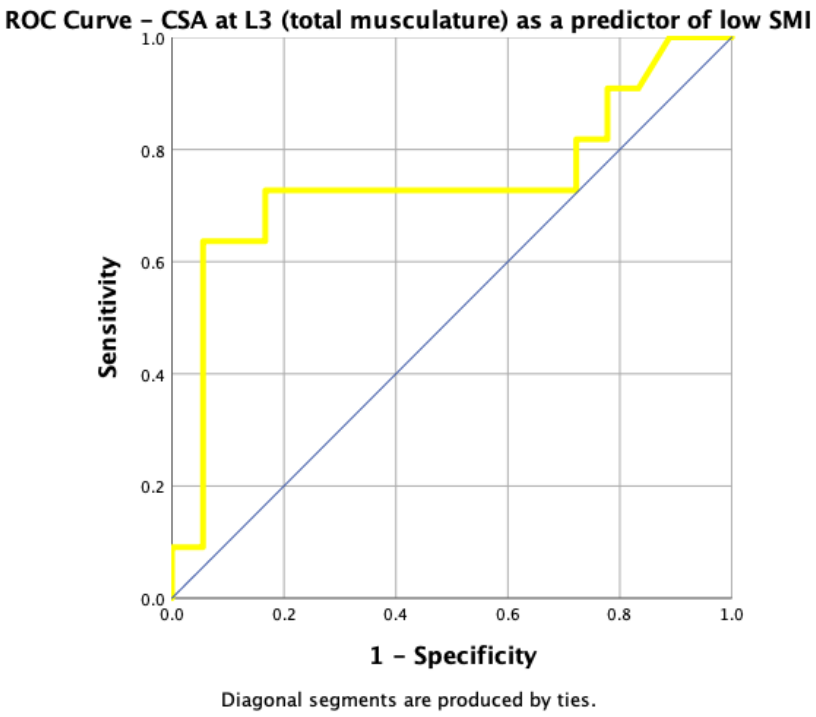


Figure 16. Receiver operator curve of cross-sectional area of total abdominal musculature at L3 as a predictor of low skeletal muscle index

The agreement between the CT metrics at the 25th and 50th percentile cut-offs and low SMI and sarcopenia was assessed using Kappa scores and is outlined in Table 11. The greatest agreement in testing occurred between the 25th percentile CSA_{AbdoL3} and low SMI, followed by the 50th percentile of CSA_{AbdoL3} and sarcopenia. Both Kappa values suggest modest agreement between the test modalities.

Table 11. Kappa statistics testing for the agreement between CT metrics of muscle mass at 2 cut-offs and sarcopenia, skeletal muscle index below gender-stratified thresholds

Comparators	Kappa	p-value
CSA L3 Psoas - 25%ile vs. Low SMI	0.054	0.76
CSA L3 Psoas - 50%ile vs. Low SMI	0.096	0.8
CSA L3 Total - 25%ile vs. Low SMI	**0.53**	**0.003**
CSA L3 Total - 50%ile vs. Low SMI	**0.37**	**0.04**
CSA L3 Psoas - 25%ile vs. Sarcopenia	0.11	0.55
CSA L3 Psoas - 50%ile vs. Sarcopenia	-0.13	0.44
CSA L3 Total - 25%ile vs. Sarcopenia	0.31	0.096
CSA L3 Total - 50%ile vs. Sarcopenia	**0.44**	**0.004**

4.5 Discussion

In our prospective cohort of kidney/kidney-pancreas transplant recipients, the level of agreement between muscle mass assessment using CT scanning and other clinical measures of muscle mass and sarcopenia were compared. Cross-sectional area of the entire abdominal musculature at the level of L3 (CSA_{AbdoL3}) correlated the best with non-structural assessment of muscle (HGS) as well as other methods of muscle mass estimation ($CSA_{PsoasL3}$, SMI derived from bioimpedance analysis). CSA_{AbdoL3} also showed good performance as a predictor of sarcopenia at a cut-off of $155cm^2$ carrying a sensitivity and specificity of 83% and 74%, respectively. The good performance of CT was only demonstrable in males, however, owing to lower numbers of females with available CT scans. The reliability of CT in female transplant recipients remains unexplored in this population. On the other hand, QMLT did not significantly correlate with any of the metrics recommended for assessment of sarcopenia by the EWGSOP2 criteria.

The importance of sarcopenia has emerged in the context of solid organ transplants outside of kidneys. Hsu *et al* (2019) showed that sarcopenia in lung transplant recipients, defined as a psoas CSA indexed to height, significantly predicted 1, 2, 3, and 4 year mortality.[10] To derive normative values, gender-based cut-offs were defined from their internal cohort based on scatterplot smoothing and spline modeling. van Vugt *et* al (2016) demonstrated in a systematic review and meta-analysis of liver transplant recipients that sarcopenia independently increased the risk of both wait-list and post-transplant mortality.[11] Based on growing reports of the influence of sarcopenia in non-renal transplant populations, the importance of being able to identify sarcopenia in the renal population becomes evident.

The impact of sarcopenia in renal populations has been highlighted in the literature. Lai *et al* (2019) described a sarcopenia prevalence of 49% in a prospective cohort study of 77

CKD patients, with half requiring renal replacement therapy in the form of dialysis.[12] They found that sarcopenia was significantly associated with increased intima-media thickness, a marker of atherosclerosis and cardiovascular risk and flow-mediated dilation of the brachial artery, a measure of endothelial dysfunction. Montreal Cognitive Assessment (MoCA) scores were significantly lower in the sarcopenic group and depression was more prevalent, suggesting a link between sarcopenia and cognitive decline and depression. These authors defined sarcopenia using HGS and Janssen's SMI[12], similar to our current study. Our prevalence of sarcopenia was much less than that reported by Lai and colleagues, possibly due to the older age of their cohort (mean age 69). Despite divergent demographics, their findings suggest that sarcopenia may be an important factor to screen for in the ESRD/transplant population, beyond the concerns of perioperative/post-transplant outcomes.

Kittiskulnam *et al* (2017) found in a prospective study that sarcopenia *per se* did not significantly impact the risk of mortality in a maintenance hemodialysis population of 645 patients, nor did any measurement of muscle mass based on bioimpedance spectroscopy.[13] Functional measures of muscle, including gait speed and grip strength (components of sarcopenia case definitions) did, however, significantly impact survival. The addition of any muscle mass covariates did not improve the predictive value of functional measures. These results emphasize the importance of functional measures of muscle. Giglio *et al* (2018), in an observational cohort study of 170 maintenance hemodialysis patients measuring muscle mass with BIA, found lower muscle strength and sarcopenia significantly increased the risk of experiencing hospitalization (aRR = 1.92 and 2.08, respectively) as well as overall survival (aRR = 1.84 and 2.09, respectively).[14] These authors maintained case definitions of sarcopenia congruent with EWGSOP2 criteria, utilizing appendicular skeletal muscle mass and HGS as defining criteria, however gait speed was not included. Lower muscle mass in isolation, however, was not significantly associated with either overall survival or hospitalization.

Hand grip strength in our cohort significantly correlated with another parameter of sarcopenic assessment: walking time. Additionally, HGS showed significant correlation to $CSA_{L3Psoas}$ and $CSA_{L3total}$. Functional measures may seemingly offer cheaper and more accessible solution to screening for sarcopenia in the clinical setting than more expensive cross-sectional imaging. Chan *et al* (2019) longitudinally studied 128 kidney transplant recipients who were ≥1 year post transplant with a functioning graft, placing patients into a sarcopenic or non-sarcopenic group. The authors defined sarcopenia by the presence of both low HGS and low muscle mass based on population normative values provided by the manufacturer of the BIA machine used in their study. They found that at a median follow up of 64 months, low strength was independently associated with their composite endpoint of mortality or hospitalization, while neither low muscle mass nor sarcopenia were significantly predictive of these outcomes.[15] The superior performance of grip strength supports the need for clinical testing of muscular function in this population. The apparent lack of prognostic significance seen by Chan *et al*. in relation to muscle mass is challenged by the case definitions of low muscle mass and sarcopenia. Their surrogate measures of muscle mass were not derived from regression equations validated in the literature, nor were the EWGSOP2 or any other published criteria used to assess sarcopenia.

The importance of standardizing or defining thresholds for muscle mass, as well as uniform agreement on case definitions of sarcopenia, are important. This was highlighted by Lamarca *et al* (2014) in a multicentre cross-sectional study, where a wide range of sarcopenia prevalence was found depending on the modality and cut-off implemented. With BIA + HGS derived case definitions, prevalence ranged from 12.7% to 45.1% with the BIA cut off at 20^{th}%ile and 2 S.D. below young population norms, respectively.[16] The BIA formula used to derive skeletal muscle mass estimations was not described, limiting the ability to confidently compare these authors' findings to our own and others in the literature.

Whole body cross-sectional imaging is considered the gold-standard referent for measuring muscle mass[6], but in its stead single slice images have been used in several research settings. One area of interest in cross-sectional imaging has been the psoas muscle as well as more expanded views of the abdominal musculature.[10, 17, 18] We chose to examine both the psoas CSA and entire abdominal muscle CSA to explore the relative performance of both metrics.

Morrell *et al* (2016) interrogated the performance of cross-sectional imaging in maintenance hemodialysis patients to predict low muscle mass. They used MRI at the level of the mid-thigh and the L4-5 level compared to whole body lean mass derived from DEXA measurement. Overall, total CSA of the mid-thigh was the greatest predictor of whole body lean mass, followed by psoas muscle cross-sectional area.[18] Furthermore, these authors demonstrated that mid-thigh muscle CSA was highly correlated with psoas CSA at L4-5 (r=0.83), and the AUC for psoas CSA for predicting sarcopenia was between 0.81 and 0.92. Sarcopenia was defined using two different percentile cut-offs based on their cohort DEXA estimations of lean body mass, rather than EWGSOP2 definitions, which rely on consideration of some measure of muscle function in addition to mass. Nevertheless, Morrell and colleagues show that psoas muscle CSA at L4-5 in the dialysis population was predictive of total muscle mass.

Our results, however, did not support such a strong relationship between $CSA_{PsoasL3}$ and total muscle mass. In fact, our data suggest that a relatively poor discriminatory capacity for psoas CSA to predict overall muscle mass with an AUC of 0.48. The reasons for this discrepancy are several-fold. Firstly, we assessed psoas CSA at the L3 level which is higher than the level studied by Morrell *et al*. This is relevant when one considers the mean CSA of the psoas in our population was 19.9cm^2 (22.6cm^2 males; 14.2cm^2 females) while the mean CSA of the psoas in the report by Morrell was 10.4cm^2. The difference between measuring at L3 vs L4-5 may have accounted for this variation, as the psoas tapers in circumference as it passes inferiorly to insert onto the lesser trochanter of the

femur. Whole body muscle mass was assessed in our study using regression equation based off BIA parameters validated in the literature for the general population with recommended cut-offs from the EWGSOP[19, 20], whereas Morrell set cut-offs of the 10th and 25th percentiles of total lean body mass based on DEXA scans. Assessing the performance of any single slice image is clearly dependent on consistent case definitions.

Shen, *et al* (2004) identified that in healthy subjects, the cross-sectional area of the entire abdominal musculature at a position of 5cm above the L4-5 level (approximately L3), was highly correlated with measures of total skeletal muscle volume using standardized whole body scans (Pearson correlation, r = 0.924).[21] When other covariates were added into a multivariable prediction model, the addition of sex, age, BMI, and waist circumference only improved the R^2 by 2.3-3.3%, suggesting the single-slice image may be used to represent total body skeletal muscle reliably.[21]

Giglio *et al* (2019) looked at patients with chronic kidney disease (of which 70% were stage 3-4) using CT to derive CSA at the L3 level of the entire abdominal musculature and SMI using bioimpedance measures (using the Janssen formula). They found a Kappa of 0.41 and AUC of 0.70 (0.60-0.81), with r = 0.51 (Pearson correlation) in males comparing these two measurements.[7] These results compare favorably to the reported outcomes of the current study. Our population of end-stage renal patients undergoing transplant demonstrated CSA_{AbdoL3} provided an AUC of 0.74 (0.53-0.95), with r=0.51 on Pearson correlation. The concordance of this CT metric in both the pre-dialysis CKD population and ESRD population undergoing transplant supports the validity of this form of testing in renal populations, and supports the utility of CT-assessed muscle mass across several stages of renal disease.

Quadriceps muscle layer thickness, an emerging measure of muscle mass in the literature, correlated poorly with functional components and structural components of sarcopenia

assessment (Table 9). The poor performance of QMLT as a predictor of sarcopenia was explored in Chapter 3. With minimal correlation between QMLT and $CSA_{PsoasL3/AbdoL3}$, the enthusiasm to further explore this metric as a surrogate for sarcopenia becomes tempered. This finding is contrasted by other studies demonstrating high concordance between mid-thigh muscle area and psoas CSA.[18] An important differentiator is that QMLT measures the thickness of the anterior compartment of the thigh (rectus femoris, vastus intermedius muscles), while other studies correlating the thigh and psoas have utilized cross-sectional area.[18] Previous studies have demonstrated concordance of QMLT and psoas CSA on CT, however. This positive relationship has been demonstrated in the young male critically ill population, with poor correlation in critically ill females and the elderly.[23] Thus, QMLT may have selective applicability to identify clinically vulnerable subpopulations. Expanded study of the QMLT in concert with other accepted metrics of sarcopenia are needed to better define this role.

Another consideration that both total CSA and muscle compartment thickness do not take into account is the quality of the muscle, namely intramuscular fibrosis or adiposity from disuse/disease and other derangements of muscle architecture.[22] Fukuda *et al* (2018) retrospectively assessed 41 pancreas transplant recipients with CTs done at the time of transplant for the presence of sarcopenia defined as a psoas muscle index (cross-sectional area indexed to height) at the level of the umbilicus below the lowest quantile in the cohort, and found that sarcopenia did not worsen the risk of complications or graft survival.[17] These authors also assessed intramuscular adipose content on the same CT scans, and found that adipose content of the multifidus muscles significantly increased the odds of complications. The adipose content was only moderately associated with psoas muscle index ($r = -0.51$). Taken together, the relative importance of muscle quality, over and above the gross size in the form of cross-sectional area or volume, may underpin the imperfect relationship between sarcopenia and CT-derived metrics in the present study. In addition, the distinction of muscle quality may be a more telling metric to consider in future research.

This study does have significant limitations that require consideration. CT scans were not available for every patient, limiting our sample size for analysis. Systematic bias around those with and without indications for CT are not able to be accounted for because of this. Additionally, the date of CT scan and transplant were inconsistent across the cohort, introducing uncertainty to the findings, as the effect of time on changes to muscle mass are not accounted for due to lack of serial imaging. Paris *et al* (2017) found that QMLT was well correlated with CT derived measures of the psoas muscle in the critically ill when the QMLT and CT were done an average of 1 day apart.[23] Bioimpedance analysis also carries inherent flaws as the values derived from BIA are influenced by hydration status, a significant consideration in the mostly dialysis dependent population undergoing transplant. Due to logistical constraints, timing of BIA was not standardized relative to dialysis runs and therefore may introduce variation in the results not able to be accounted for. On balance, a strength of our study is that we maintained validated case definitions of sarcopenia and cut-off values from the sarcopenia literature. This is also the first report to the authors' knowledge of assessing metrics of sarcopenia/muscle mass in the ESRD population at the time of transplant.

In summary, CT_{AbdoL3} demonstrated good predictive ability to identify sarcopenia. Further exploration of this metric in the kidney transplant population is warranted to better characterize population normative values as well as gender-specific thresholds to define low muscle mass for standardization in future studies. These measurements should be maintained within the context of muscular function, as is suggested by consensus guidelines on defining sarcopenia. With improved definitions to operationalize sarcopenia, the impact on clinical outcomes can be better studied and appreciated and opens the door to rational interventions to impact populations at risk.

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5 General Conclusions and Future Directions

The impact of frailty and sarcopenia in renal transplant recipients has been established in the literature, and is continuing to be refined over time. The literature has demonstrated that frailty, assessed using the Physical Frailty Phenotype, significantly affects recipient's LOS, DGF rates, and influences survival, thus validating its use in the transplant population.[1-3] Sarcopenia is intimately related to frailty, acting as either a component of the underlying pathophysiology of frailty or another manifestation of a common pathway with frailty.

The QMLT has received attention in several contexts as an accessible surrogate measure of frailty and/or sarcopenia that appears to be reliable in published reports.[4-7] Our use of the QMLT identified those at risk of longer LOS after transplant. It is possible that with greater numbers of participants to allow stratification by donor type, statistical power can be maintained to refine the influence of this metric on patient outcome. Further study of the QMLT in the transplant population should be undertaken to expand on this finding, and to refine the value of measuring the QMLT. We intend to expand on our number of participants. QMLT in isolation did not show good discrimination for the assessment of sarcopenia but was correlated with overall skeletal muscle mass using bioimpedance derived calculations. It is possible this observation relates to the functional assessment that underlies sarcopenia that is not captured by QMLT, which is purely quantitative. While QMLT may not be a perfect tool as a one-time assessment, its value as a serial measurement warrants exploration, as QMLT has shown reliability to detect changes to resistance training over time[11], and thus perhaps the dynamics of QMLT may be more telling than a single absolute value.

Muscle quality is another factor that deserves future study. All assessments in the present study relied on gross muscle size estimations and clinical functional testing, but no assessment of muscle architecture was included. Intramuscular adipose content, fibrosis, density of mitochondria, exhaustion of muscle satellite cells, and degradation of motor end plates could all represent significant factors that better inform a patient's underlying degree of sarcopenia/frailty.[8, 9] These variables are not necessarily appreciable with standard cross-sectional imaging, strength testing, and other available measurement tools in clinical practice. Correlation between histologic and biochemical parameters of skeletal muscle with currently available assessment tools, such as muscle density on cross-sectional imaging, warrants exploration in the future.

It was seen in this study that cross-sectional area of all abdominal muscles at the level of L3 had the greatest discrimination for sarcopenia. This finding is limited to males, as there were insufficient females with complete data to allow for analysis. The variable timing in administration of CT scans also possibly confounds our results. Future studies should look to validate this CT metric by examining recipients' muscle mass in a systematic and controlled fashion to improve the validity of this metric in this population, and to refine normative values. The impact of CT measurements of muscle on clinical outcomes would then be considered with greater confidence. We hope to continue to accrue patients in an ongoing effort to augment the number of patients with CT scans available for assessment, however ethics board standards limits the ability to expose potential recipients to CT scan radiation without clinical justification which could hinder future numbers as it did in the present cohort. Regardless, greater numbers of CT-derived metrics will allow for examination of these values and the relationship to clinical outcomes, similar to the way QMLT was studied here.

Lastly, consensus on simplifying the definition of sarcopenia in the renal failure and transplant population should be sought: current application of operational criteria for sarcopenia in this population remains theoretical.[10] This would achieve improved

standardization of research protocols to allow confidence in comparing outcomes, and would provide basis to help classify patients accurately in the clinical context for risk stratification and intervention.

The impact of frailty and sarcopenia on outcomes of renal transplant are maturing and gaining importance for consideration in the assessment of potential transplant recipients. As the prognostic impact of these metrics is further clarified, an impetus to incorporate formal testing into the assessment process for prospective transplant recipients arises. The multitude of frailty instruments and different ways to assess the components of sarcopenia potentially leaves clinicians and assessment programs at an impasse over which method would be the most feasible and informative. The QMLT was assessed as a potential surrogate for frailty and sarcopenia with the potential to be a single-step assessment that could simplify the multifaceted testing of frailty and sarcopenia. This report supports a potential role for the QMLT, but its validity remains to be confirmed, and other forms of assessment such as CT of the abdomen along with possibly expanded appendicular muscular assessment with ultrasound require further exploration to identify valid cut-offs for clinical prognostication. The goal of finding a simple, feasible and meaningful screening metric remains to identify those at greatest risk of complications. Identifying such individuals would ideally lead to improved risk counselling and potentially allow for invitation of these patients into therapeutic programs that may involve prehabilitative exercise, pharmacotherapy, or dietary interventions that could alter the progression of frailty and sarcopenia.

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Appendices

Appendix 1. Frailty phenotype data collection sheet and cut-off references for strength, walk time, activity levels, exhaustion, weight loss

From: https://www.cgakit.com/fr-1-frailty-phenotype

FRAILTY PHENOTYPE

NAME _____

d.o.b. _____

Date _____

Administered by _____

CRITERIA	OPTIONS	WEIGHT	SCORE
Unintentional weight loss	no	0	
	yes	1	_____
Physical Activity	Not limited or little limited	0	
	Limited a lot	1	_____
Low resistance/exhaustion	0 to 2 days	0	
	3 to 7 days	1	_____
Strength	< 20% weaker	0	
	> 20% weaker	1	_____
Walking Time	Not slower	0	
	Slower	1	_____
		TOTAL SCORE	_____

SCORING

0 = robust
1-2 = pre-frail
3-4 = frail
5 = very frail

NOTES :

1

DEFINITIONS

Unintentional weight loss :
>5% weight loss over past year
or
>4,5 Kg weight loss over past year

Physical Activity :
Health imposes a limit on vigorous activities such as, mowing the lawn, raking, gardening, hiking, jogging, biking, exercise cycling, dancing, aerobics, bowling, golf, swimming, other sport.

Low resistance/exhaustion :
Frequency that, in the past week, the individual felt that everything s/he did was an effort
or
s/he could not "get going"

Strength :
Without dynamometer :
Estimated 20% weaker than expected in an individual of similar size (BMI)
or
With dynamometer (stratified by gender and Body Mass Index quartiles)

Men	*Cutoff for grip strength (Kg) criterion for frailty*
BMI < 24	< 29
BMI 24.1–26	<30
BMI 26.1–28	< 31
BMI > 28	<32
Women	*Cutoff for grip strength (Kg) criterion for frailty*
BMI < 23	< 17
BMI 23.1–26	< 17,3
BMI 26.1–29	< 18
BMI > 29	<21

Walking Time :
(stratified by gender and height)

Men	*Cutoff for Time to Walk 15 feet criterion for frailty*
Height > 173 cm	6 seconds
Height < 173 cm	7 seconds
Women	*Cutoff for Time to Walk 15 feet criterion for frailty*
Height > 159 cm	6 seconds
Height < 159 cm	7 seconds

Curriculum Vitae

Name: Max Alexander Levine

Post-secondary Education and Degrees:

University of Alberta
Edmonton, AB, Canada
2005-2009 B.Sc. (Kinesiology)

University of Alberta
Edmonton, AB, Canada
2009-2013 M.D.

University of Alberta
Edmonton, AB, Canada
2013-2018 – F.R.C.S.C in Urology

Western University
London, Ontario, Canada
2019-2020 M.Sc. (Surgery) – in progress
2018-2020 AFC diploma in Solid Organ Transplant - in progress

Honours and Awards:

Canadian Graduate Scholarship (CGSM)
Canadian Institute of Health Research
2019-2020

Eric Shepherd Travel Award for Transplant Research
Western University
2019

Frank Squires Scholarship in Urology
University of Alberta
2017

Related Work Experience

Resident Physician - Urology
University of Alberta
2013-2018

Clinical Fellow – Kidney/Pancreas Transplant
2018-2020

Recent Publications:

1. Levine, M, Luke P, Sener A. (2020). Canadian survey on the use and justification for intraoperative diuretics during renal allograft reperfusion. Canadian Journal of Surgery. In Press
2. Aquil S, Olvera-Posada D, Navaratnam R, Mikhail D, Levine MA, Luke PP, Sener A. (2020). Comparative Study Assessing Postoperative Renal Loss Using Two Different Partial Nephrectomy Techniques: Off- Clamp versus Standard On-Clamp Surgery. Current Urology. 20
3. Wilson C, Chahine S, Cristancho S, Aquil S, Mandurah M, Levine M, Sener A. (2019). Unusual suspects: Real time physiological evaluation of stressors during laparoscopic donor nephrectomy. Canadian Urologic Association Journal. Submitted,
4. Levine M, Schuler T, Gourishankar S. (2017). Complications in the 90-day postoperative period following kidney transplant and the relationship of the Charlson Comorbidity Index. Canadian Urological Association Journal. 11(12): 388-93.
5. Kinnaird A, Levine M, Ambati D, Zorn J, Rourke K. (2014). Stricture length and etiology as preoperative independent predictors of recurrence after urethroplasty: A multivariate analysis of 604 urethroplasties.Canadian Urologic Association Journal. 8(5-6): E296-300.

6. Levine M, Kinnaird A, Rourke K. (2014). Revision urethroplasty success is comparable to primary urethroplasty: a comparative analysis.Urology. 84(4): 928-32.
7. Levine M, Evans H. (2013). Open removal as a first line treatment of magnetic intravesical foreign bodies. Canadian Urologic Association Journal. 7(1): E25-28.