Identifying cachexia and sarcopenia associated risk in gastrointestinal and hepato-pancreato-biliary surgery

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Dissertation

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General Introduction

GENERAL INTRODUCTION

Disorders characterised by unwanted weight loss, also known as wasting disorders, were first reported by physicians on the island of Kos in ancient Greece. Hippocrates described it vividly in his works [1].

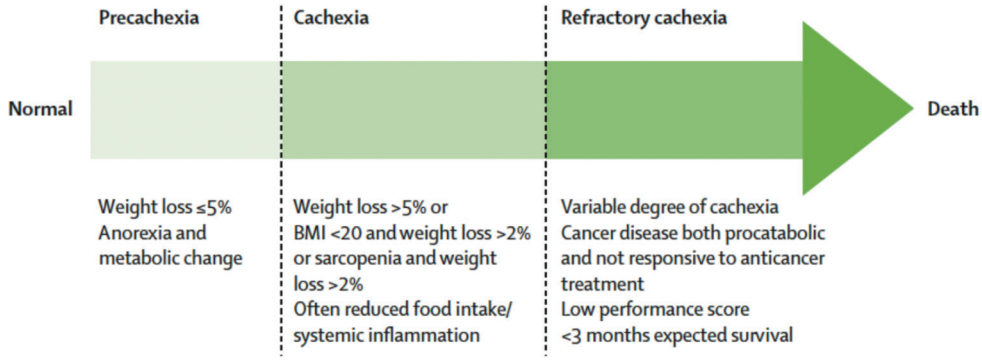
> "*The flesh is consumed and becomes water, the abdomen fills with water, the feet and legs swell, the shoulders, clavicles, chest and thighs melt away. This illness is fatal.*"
>
> Hippocrates (c. 460-377 BC)

Through the course of history, the condition of involuntary weight loss became known as consumption, which eventually became synonymous with tuberculosis due to the excessive weight loss associated with that disease. This condition became known as cachexia, which etymologically finds its origins from the Greek, *kakos* and *hexix,* meaning 'bad condition'. Today, cachexia is defined according to an international consensus definition as a multifactorial syndrome characterised by involuntary progressive weight loss as a result of a reduction of skeletal muscle mass, with or without the depletion of adipose tissue [2, 3]. The breakdown of muscle tissue is known as sarcopenia, the name of which is derived from the Greek word *sarx* meaning flesh, and *penia* meaning poverty. This condition is clinically defined as a loss of muscle mass and / or muscle strength and is the main clinical feature of cachexia [4, 5]. Although sarcopenia is primarily a disease of the elderly [6], its development is also associated with conditions that are not exclusively seen in the elderly, such as disuse, malnutrition, and cancer [7]. Not all patients suffering from sarcopenia are classified as being cachectic, as not all patients suffering from sarcopenia display weight loss [5, 7]. It is therefore not always possible to distinguish between these two interrelated wasting disorders.

Defining cachexia

Cachexia comprises a spectrum of symptoms ranging from precachexia, characterised by mild weight loss and anorexia, to cachexia, which is associated with more severe weight loss, sarcopenia, and systemic inflammation, and finally refractory cachexia where there is no more response to anti-cancer treatment and life expectancy is less than three months [8]. Prior to reaching its refractory phase, cachexia is not completely irreversible. Often, reduced food intake can be treated through the management of symptoms affecting appetite (for example, uncontrolled pain or constipation) [9] or with appetite stimulants or nutritional support [10, 11]. However, these potentially reversible elements of cachexia are often only minor contributors, and although improved nutritional intake can partly reverse fat loss, the metabolic changes resistant to current interventions largely prevent significant reversal of muscle wasting [10, 11].

Figure 1. Stages of cancer cachexia



Cachexia represents a spectrum through which not all patients will progress. At present, there are no robust biomarkers to identify those precachectic patients who are likely to progress or to predict the rate at which they will do so. Refractory cachexia is defined essentially on the basis of the patient's clinical characteristics and circumstances. BMI=body-mass index. Image from Fearon et al., Lancet Oncology 2011 [8].

Pathogenesis of cancer cachexia

The pathogenesis of cancer cachexia is multifactorial and characterized by a negative protein and energy balance that is driven by a combination of reduced food intake and abnormal metabolism [8]. Concurrent hypermetabolism, hypercatabolism, and hypoanabolism aggravate weight loss and are thought to be provoked by tumour induced systemic inflammation and catabolic factors that can act partially via the central nervous system [2].

Muscle atrophy occurs when the overall rate of protein degradation exceeds the protein synthesis rate. Studies in animal models of cancer cachexia suggest that the ubiquitin-proteasome (UPS) pathway plays an important role in the degradation of myofibrillar proteins, although this is still controversial in cancer patients [12-15]. Skeletal muscle catabolism has been shown to be induced by both tumor- and host-derived factors. These factors have been primarily identified through *in vitro* experiments and tumor xenograft models, and include pro-inflammatory cytokines and members of the transforming growth factor-β (TGF-β) superfamily, including activins, myostatin, and TGFβ, and pro-inflammatory mediators that promote skeletal muscle catabolism, which include interleukin-6 (IL-6), IL-1, tumor necrosis factor alpha (TNF-α), IFN-γ, leukemia inhibitory factor (LIF), and growth/differentiation factor 15 (GDF15) [16-20].

Myosteatosis

Fat can accumulate in skeletal muscles in the form of intramyocellular lipid droplets within the cytoplasm of myocytes and/or as intermuscular adipocytes [21]. In physiological situations, these lipid stores are thought to provide fuels for skeletal muscle contraction and

vary with aerobic fitness levels and sensitivity to insulin. The amount of intramyocellular stores can be altered through short-term dietary interventions where fat intake is varied, as well as through resistance training [22, 23]. Myosteatosis is characterized by increased inter- and intra-myocellular fat stores, and, although its pathophysiology is still unclear, has been shown to be associated with insulin resistance and systemic inflammation [22, 24-29]. Myosteatosis is relatively poorly characterized. However, a clinical relationship between myosteatosis and impaired physical function, poor short-, and long-term oncological outcome, and delayed recovery after surgery has been clearly documented [27, 30-37].

Cachexia and oncological outcome

It is well established that cachexia is responsible for the death of at least 20% of all cancer patients [38]. The incidence among cancer patients varies according to cancer type, with indices of up to 80% for gastric and pancreatic cancer patients, 50% for patients with lung, colon, or prostate cancer, and around 40% for patients with breast cancer or leukemia [38, 39]. Separating cancer-induced cachexia from the effects and complications after cancer therapy is often difficult. Muscle loss can be induced by surgery, [40] cytotoxic chemotherapy, [41] androgen-deprivation therapy [42], and targeted therapies that potentially interfere with pathways of muscle anabolism [43]. Irrespective of the cause, sarcopenia has been shown to have significant negative impact on short- and long-term outcome following a range of oncological treatments [38, 44-51].

Postoperative morbidity

Not only long-term outcome has been shown to be impacted by cachexia and sarcopenia. Postoperative complications (morbidity) have profound impact on the burden of disease and suffering following surgical treatment [45, 46, 52-55]. Pulmonary morbidity, especially postoperative pneumonia, is of particular importance and has been shown to prolong hospital admission and increase in-hospital mortality following a range of surgical interventions, most notably after major abdominal and upper gastro-intestinal surgery [56-60]. More specifically, patients undergoing partial hepatectomy frequently develop reactive pleural effusion, which increases the risk of post-operative pneumonia to incidences above 10% [56, 58-63]. Besides the medical implications, pulmonary complications constitute a significant burden to healthcare systems by increasing healthcare costs [58, 61].

Pulmonary function and sarcopenia

Sarcopenia has been shown to be associated with reduced pulmonary function in healthy adults, as well as with increased pulmonary complications following abdominal surgery [64-68]. Interestingly, very little is known about thoracic muscle quality or function of the diaphragm in the context of sarcopenia and wasting disorders [69, 70]. Some preclinical

animal studies have shown that sarcopenia is associated with atrophy of diaphragmatic muscle fibers, and that ageing is related to a decline in diaphragmatic function [71-73]. Clinical studies in the context of prolonged mechanical ventilation of acutely ill patients have focused on diaphragm function using ultrasound technology and have shown that prolonged ventilation can lead to diaphragm atrophy which is associated with worse clinical outcome [74-76].

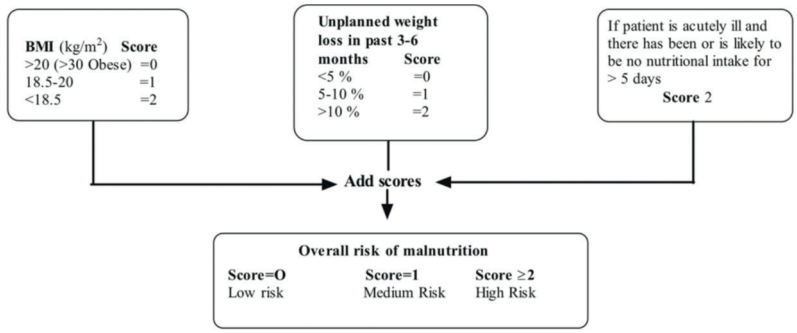
Screening and early detection

The best available diagnostic feature of cancer cachexia is involuntary weight loss, making screening for weight loss and monitoring of body weight over time an important clinical assessment [77]. Early detection of weight loss is of paramount importance for the implementation of treatment for patients who are at risk. Several tools, ranging from easy-to-use questionnaires to more complex computed tomography (CT) image analysis have been developed to identify patients who are at risk for, or display symptoms associated with these conditions. For the purpose of this thesis, we will elaborate on the different tools which are commonly used to identify wasting disorders in both the clinical and academic setting. We will also describe some experimental methods not previously described.

Nutritional risk screening tools

The Global Leadership Initiative on Malnutrition (GLIM) defines an international consensus for the identification of malnutrition [78]. The GLIM criteria state that a combination of at least one phenotypic and one etiological criterion must be met in order for the diagnosis of malnutrition to be made. Phenotypic criteria include unwanted weight loss, low body mass index (BMI), reduced muscle mass, or reduced food intake. The etiological criterion consists of disease burden and / or inflammation. Nutritional risk screening tools are efficient and easily implementable screening tools for the detection of nutritional risk that are widely used in the clinical setting. Different countries, regions, and medical disciplines use a range of different screening tools, which all find common ground in their composition. In the context of this thesis, we will elaborate on two commonly used screening tools, which are recommended by the European Society for Nutrition and Metabolism (ESPEN), namely the MUST (Malnutrition Universal Screening Tool) and the NRS-2002 (Nutritional Risk Screening Tool 2002) [79, 80]. These questionnaires include simple questions regarding weight loss, appetite, and severity of disease, as well as the determination of BMI, to create a score. A higher score constitutes an increased risk of malnutrition or undesired weight loss. The MUST (figure 2) is the more compact of the two, and scores BMI, weight loss in the past 3-6 months, and acute disease effect. A score of 2 or higher indicates an increased nutritional risk, which warrants the implementation of a nutritional intervention.

Figure 2.



Malnutrition Universal Screening Tool, Kondrup et al 2002

The NRS-2002 (figure 3) is the more complex of the two. It is comprised of a pre-screening containing 4 questions regarding BMI, weight loss, dietary intake, and illness. If any of these questions are answered affirmatively, the test proceeds. The NRS-2002 includes scores for nutritional status and weight loss, as well as severity of disease and age over seventy years. A score of three or higher indicates an increased nutritional risk score and should be followed by a nutritional intervention.

Figure 3. Nutritional Risk Screening (NRS 2002)

Table 1 Initial screening

		Yes	No
1	Is BMI <20.5?		
2	Has the patient lost weight within the last 3 months?		
3	Has the patient had a reduced dietary intake in the last week?		
4	Is the patient severely ill ? (e.g. in intensive therapy)		

Yes: If the answer is 'Yes' to any question, the screening in Table 2 is performed.
No: If the answer is 'No' to all questions, the patient is re-screened at weekly intervals. If the patient e.g. is scheduled for a major operation, a preventive nutritional care plan is considered to avoid the associated risk status.

Table 2 Final screening

Impaired nutritional status		Severity of disease (≈ increase in requirements)	
Absent Score 0	Normal nutritional status	Absent Score 0	Normal nutritional requirements
Mild Score 1	Wt loss >5% in 3 mths or Food intake below 50–75% of normal requirement in preceding week	Mild Score 1	Hip fracture* Chronic patients, in particular with acute complications: cirrhosis*, COPD*. *Chronic hemodialysis, diabetes, oncology*
Moderate Score 2	Wt loss >5% in 2 mths or BMI 18.5 – 20.5 + impaired general condition or Food intake 25–60% of normal requirement in preceding week	Moderate Score 2	Major abdominal surgery* Stroke* *Severe pneumonia, hematologic malignancy*
Severe Score 3	Wt loss >5% in 1 mth (>15% in 3 mths) or BMI <18.5 + impaired general condition or Food intake 0-25% of normal requirement in preceding week in preceding week.	Severe Score 3	Head injury* Bone marrow transplantation* *Intensive care patients (APACHE>10).*
Score:	+	Score:	= Total score
Age	if ≥70 years: add 1 to total score above	= age-adjusted total score	

Score ≥3: the patient is nutritionally at-risk and a nutritional care plan is initiated
Score <3: weekly rescreening of the patient. If the patient e.g. is scheduled for a major operation, a preventive nutritional care plan is considered to avoid the associated risk status.

Nutritional Risk Screening 2002 (NRS-2002), Kondrup et al 2002

Screening tools for the identification of sarcopenia

The European Working Group for Sarcopenia in Older People (EWGSOP) proposes a number of clinical and imaging tools for diagnosing sarcopenia [81]. The most clinically efficient methods for assessing muscle function and physical activity are described below.

Handgrip strength

Handgrip strength is a clinically easy-to-use tool for assessing muscle function and can be used to identify sarcopenia [81]. Low grip strength is a clinical marker of poor mobility and, according to some studies, it is a better predictor of clinical outcome than low muscle mass [82].

Fitness test

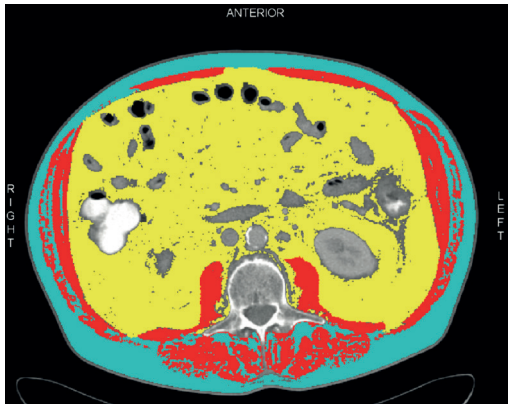
The SPPB test (Short Physical Performance Battery) is recommended by the European Working group for Saropenia in Older Patients (EWSOP) and can be carried out to assess patient's physical performance. The SPPB assesses balance, gait, strength, and endurance by examining a person's ability to stand with their feet next to each other in half and tandem positions, walk 2.5 meters and get up from a chair, and sit five times in a row. A combination of gait grip strength and gait speed can be used to reliably identify sarcopenia in elderly patients [81].

Body composition imaging

In addition to clinical screening tools, CT imaging is often used in the academic setting for the detection of sarcopenia [46, 51, 83-87]. This method uses CT images at the level of the third lumbar vertebra (L3) to determine cross-sectional area of skeletal muscle, visceral adipose tissue, and subcutaneous adipose tissue. The total areas of skeletal muscle (SM), visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT) are then corrected for stature of the patient to calculate the L3-muscle index, L3-VAT index, and L3-SAT index in cm^2/m^2, providing good estimates of total body SM-, VAT-, and SAT-mass (Figure 4) [83, 88, 89]. Several cut-off values defining sarcopenia have been published, these cut-off values vary amongst different ethnicities, but always acknowledge sex-specific body composition differences.

Besides the estimation of mass, e.g. for the detection of sarcopenia, CT image analysis can also be used for the estimation of muscle density, also known as skeletal muscle radiation attenuation (SM-RA). SM-RA is expressed as the mean value of Hounsfield units in skeletal muscle tissue and is considered to be a radiological marker indicative of myosteatosis [33-35, 90].

Figure 4. Body composition segmentation



CT image of a patient suffering from myosteatosis at the third lumbar vertebra (L3) level, with muscle and fat segmentation: red; SMA (Skeletal Muscle Area), blue; SAT (Subcutaneous Adipose Tissue) and yellow; VAT (Visceral Adipose Tissue)

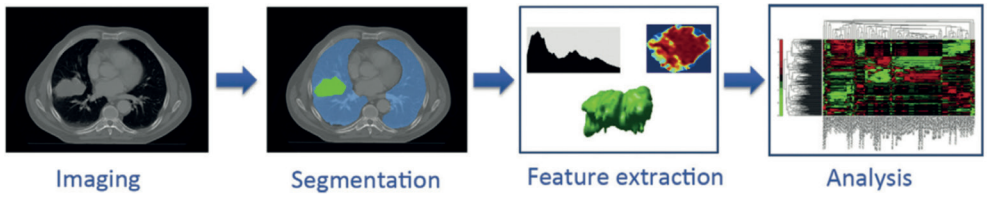
Radiomics

The field of radiomics was developed under the premise that advanced analysis of conventional and novel medical images could capture additional information not currently used. More specifically, it was suggested that genomic and proteomics patterns can be expressed in terms of macroscopic image-based features [91]. Radiomics features have been shown to provide prognostic value in predicting clinical outcomes of several tumor entities, including head and neck cancer and lung tumors [92-94]. It has been demonstrated that major differences in protein expression patterns within a tumour can be correlated to radiographic findings (or radiophenotypes) based on CT data [95]. Such data could play a beneficial role in improving treatment algorithms used for targeted agents and personalised medicine [96-98].

Process of radiomics analysis

The process of radiomics analysis starts with the segmentation of a region of interest (ROI) within the image. This can be done (semi) automatically or manually depending on the nature and location of the ROI. The next step in the process is the extraction and analysis of quantitative data (figure 5).

Figure 5.



The radiomics workflow. On the medical images, segmentation is performed to define the tumour region. From this region, the features are extracted, e.g. features based on tumour intensity, texture, and/or shape. Finally, these features are used for analysis, e.g. the features are assessed for their prognostic power. Image by Lambin et al. 2012, European Journal of Cancer [91].

Radiomics features are processed by three main characteristics, namely geometry, intensity of signal, and texture (figure 6). These features form the basis of a range of first and second order radiomics features.

Figure 6.

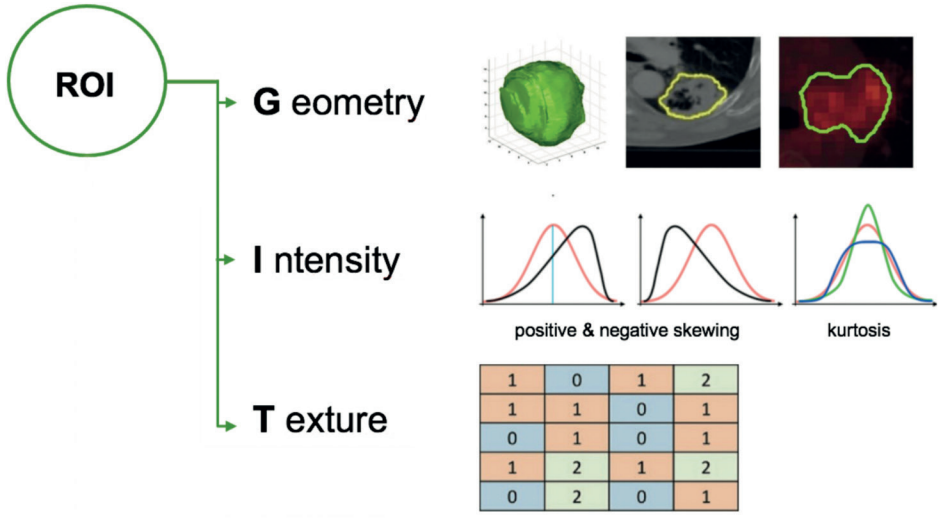


Figure showing three main categories of radiomics features; geometry, intensity, and texture.

The process of feature processing creates large amounts of quantitative data. Depending on the nature, size, and dimensions of the ROI, the number of extracted features can range in the hundreds. The reduction of these vast quantities of features to manageable numbers is of great importance for performing robust statistical analysis [91]. A number of methods, ranging from the implementation of artificial intelligence, deep learning algorithms, or correlation elimination can be performed to achieve adequate reduction of features. Following adequate feature reduction and selection, investigation of the outcome parameter of interest can be performed.

As previously mentioned, radiomics analysis was first implemented in tumor imaging analysis for the purpose of more accurate image-based tumor staging. Recently, attempts have been made to implement radiomics analysis in non-tumor tissue. De Jong et al. attempted to identify radiomics features associated with muscle wasting in a non-small cell lung carcinoma cohort undergoing chemotherapy [99]. Their work constituted the first step in exploring the use of radiomics body composition analysis and prefaces the further exploration of the potential uses of radiomics image analysis.

GENERAL AIM OF THE THESIS

The present thesis aims to:

1. Assess the efficacy of nutritional screening tools for the prediction of postoperative morbidity following gastrointestinal surgery
2. Investigate myosteatosis as a risk predictor of postoperative morbidity following orthotopic liver transplantation
3. Determine the potential of using thoracic myosteatosis for the identification of patients at risk for postoperative pneumonia following the resection of hepatic colorectal metastasis
4. Investigate the effect of major liver resection on diaphragm function in sarcopenic and non-sarcopenic patients
5. Assess whether radiomics-based analysis of body composition features can discriminate between patient groups with in- or decreased overall survival following curative resection of the pancreatic head for the treatment of Pancreatic Ductal Adenocarcinoma (PDAC)

THESIS OUTLINE

In oncology and oncological surgery, nutritional risk screening is of vital importance for the identification of malnourished, cachectic patients, and commencement of appropriate, early nutritional intervention [79, 80]. In **chapter two,** we prospectively investigated nutritional risk tools for their association with postoperative morbidity. We went on to compare nutritional risk scoring tools with more complex CT-based whole-body quantification of muscle mass in **chapter three**. Developments in the field of body composition imaging have shown that myosteatosis might be of greater predictive value for outcome than muscle mass [26, 27, 34-36]. In **chapter four,** we investigated the predictive value of myosteatosis for postoperative morbidity following deceased donor liver transplantation.

One of the most important factors contributing to postoperative morbidity and mortality is postoperative pneumonia [57, 58, 60, 61, 66, 68, 100, 101]. In **chapter five,** we prospectively investigated the association between myosteatosis at the third lumbar

and fourth thoracic vertebrae with the incidence of postoperative pneumonia. The process of investigating pulmonary morbidity led us to the analysis of the diaphragm. Interestingly, very little is known about the function of the diaphragm in the context of sarcopenia and wasting disorders, or how its function is influenced by abdominal surgery. This led us to the design of a prospective observational study, described in **chapter six,** which investigates the function of the diaphragm during the perioperative period using trans-costal ultrasound. This study will evaluate the differences in diaphragm function and recovery between sarcopenic and non-sarcopenic patients undergoing major liver resection.

We investigated the implementation of novel radiomics approaches to the field of body composition imaging in **chapter seven**. We investigate whether the quantification of large amounts of body composition imaging data could provide added value for the identification of patients at risk for reduced survival following oncological pancreatic resection.

In **chapter eight,** we will broadly summarize and discuss the obtained results in view of previous data, and in **chapter nine** we discuss the impact of our research on patients, the healthcare system and research.

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CHAPTER

2

Evaluation of nutritional status as an independent predictor of morbidity following colorectal surgery

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ABSTRACT

Background: Nutritional Risk Screening-2002 (NRS-2002) and the Malnutrition Universal Screening Tool (MUST) are screening tools for nutritional risk. These screening tools have also been used to predict post-operative complications and morbidity, however, not all studies confirm the reliability of nutritional screening for the prediction of postoperative morbidity. Our study aims to evaluate the independent predictive value of nutritional risk screening in addition to currently documented medical, surgical and anesthesiological risk factors for post-operative complications, as well as length of hospital stay.

Methods: This study is a prospective observational cohort study of 129 patients undergoing elective gastro-intestinal-surgery. Patients were screened for nutritional risk upon admission using both MUST and NRS-2002 screening tools. Univariate and multivariate analyses were performed to investigate the independent predictive value of nutritional risk for post-operative complications and length of hospital stay.

Results: MUST ≥2 (OR 2.87; 95% CI 1.05-7.87) and peri-operative transfusion (OR 2.78; 95% CI 1.05-7.40) were significant independent predictors for the occurrence of post-operative complications. Peri-operative transfusion (HR 2.40; 95% CI 1.45-4.00), age ≥70 (HR 1.50; 95% CI 1.05-2.16) and open surgery versus laparoscopic surgery (HR 1.39; 95% CI 0.94-2.05) were independent predictors for increased length of hospital stay, whereas American Society of Anesthesiology Score (ASA) and MUST were not.

Conclusion: Nutritional risk screening (MUST ≥2) is an independent predictor for post-operative complications, but not for increased length of hospital stay.

INTRODUCTION AND RATIONALE

In Western Europe approximately 30% of all patients admitted to hospital are undernourished [1, 2]. During hospital stay the nutritional status of patients has been shown to decline further [3, 4]. Malnutrition has been shown to impair immune function, delay wound healing and convalescence from illness, and to decrease functional status [5].

Several screening tools have been developed for recognizing nutritional risk [6]. European Society for Nutrition and Metabolism (ESPEN) guidelines recommend a screening tool for in-hospital use, called the NRS-2002 (Nutritional Risk Screening Tool 2002) [1]. In the Netherlands however, the NRS-2002 is not widely implemented due to its more time consuming questionnaire. Instead, more compact screening tools, such as the MUST (Malnutrition Universal Screening Tool) and SNAQ (Short Nutritional Assessment Questionnaire) have been widely implemented [7].

Nutritional screening tools were developed and validated to recognize nutritional risk and evaluate therapeutic effect.[6] The NRS-2002 has also been used to predict post-operative complications and morbidity, though not all studies confirm the reliability of nutritional screening for this purpose [8-11]. Our study aims to evaluate the independent predictive value of nutritional risk screening for post-operative complications after major gastro-intestinal surgery, as well as for length of hospital stay, in addition to currently documented medical, surgical and anesthesiological risk factors.

Study design & methods

This study is a prospective observational cohort study of patients undergoing elective surgical intervention at VieCuri teaching hospital in the south of the Netherlands. Patients aged 18 years and older undergoing elective surgery to the gastro-intestinal tract between October 2012 and July 2013 were eligible for participation. We opted to include patients undergoing major gastro-intestinal and colorectal surgery, thus patients undergoing appendectomy and cholecystectomy were not eligible for participation (Table 1). One hundred thirty-eight patients were approached for participation, of whom 9 refused enrolment in the study. Exclusion from participation was on the basis of an ASA-classification V, severe liver cirrhosis – Child grade C, end stage renal disease requiring dialysis, severe heart disease – New York Heart Association class IV and chronic obstructive pulmonary disease (COPD) requiring (home)oxygen therapy (N=0).

Prior to this study a nutritional screening and intervention algorithm had successfully been implemented at our hospital in accordance with ESPEN guidelines. All Patients were screened using the MUST screening tool upon evaluation upon hospital admission. For the benefit of this study the NRS-2002 was used alongside the MUST [1]. Patients with MUST scores of 2 or higher and/or NRS-2002 scores of 3 or higher received nutritional intervention before surgery, in accordance with ESPEN guidelines. As a nutritional

screening and treatment algorithm was already in place no change in the nutritional intervention regime was implemented in this study.

Table 1. Type of surgery

Operation	**Frequency n (%)**
Hemi-colectomy	31 (23)
Colo-ileostomy reversal	25 (19)
Rectum resection/ amputation	20 (16)
Sigmoid resection	19 (15)
Colo-ileostomy	11 (9)
Gastric surgery	10 (7)
Liver segment resection	6 (5)
Extensive abdominal surgery	5 (4)
Ileocaecal resection	2 (2)
Total	**129**

Besides nutritional status the presence of known risk factors for post-operative complications were recorded: age (<70 and ≥70), ASA-classification (<3 and ≥3), underlying disease (malignant or benign), planned type of procedure (laparoscopic or open: conversion from laparoscopic to open surgery was classified as open surgery), perioperative anemia (any deviation from normal haemoglobin ranges) and peri-operative blood transfusion. Comorbid disease was categorized according to the Charlson Comorbidity Index. In the Charlson Comorbidity index a weighted score is assigned to each of 17 comorbid conditions, the sum of the index score is an indicator of disease burden, and an estimator of mortality. For the analysis we classified the Charlson index as a binary outcome, ≥3 being an increased comorbidity risk.

The primary endpoint was post-operative complications. Post-operative complications were categorized by severity as proposed by Clavien-Dindo [12]. According to this classification, complications are defined as any deviation from the normal post-operative course. Complications were graded from I to V, based on the extent of intervention needed to correct the complication. In the case of several complications these were recorded separately, and in this case the most severe complication was used in statistical analysis. For the initial analysis we classified postoperative complications as a binary outcome: complications graded 2 or higher were categorized as the occurrence of post-operative complications, whereas grade 0 and 1 were graded as no complications. We also evaluated the influence of our study variables on an increase in complication severity, by using a proportional odds model, as described in statistical methods. This analysis selectively included grade one complications. Of grade one complications we only included wound infections opened at bedside in our study (N=7).

Length of hospital stay was the secondary endpoint and was defined as time from admission until time of discharge (in days). In our institution patients are admitted on

the day of surgery. In cases where patients were admitted a week prior to surgery for pre-operative nutritional support, the length of hospital stay was classified as time from the day of surgery until the day of discharge.

It was our goal to determine the independent predictive value of nutritional risk, and in the process create a risk-stratification for the occurrence of operative complications and increased length of hospital stay. We intended to differentiate between risk factors that are known pre-operatively and those that become apparent peri-operatively. We therefore created pre-operative multivariate model as well as a peri-operative model for both our primary and secondary outcome measures. Ethical approval was granted for this study by a local medical ethics committee.

STATISTICAL ANALYSIS

univariate analyses

Univariate analyses were used to determine the association between each individual variable and the outcome variables. The association between MUST, NRS-2002, age (<70 and ≥70), ASA-classification (<3 and ≥3), underlying disease (malignant or benign), comorbidity (Charlson Index,) type of procedure (laparoscopic or open), peri-operative anemia, peri-operative transfusion, and the occurrence of complications were tested with the chi-square method. For analyzing the association between the above-mentioned predictors and time to hospital discharge, log rank testing was performed.

Multivariate analyses

Multiple logistic regression analysis was used to analyze the independent association between patient, disease and treatment characteristics and the occurrence of post-operative complications. Cox proportional hazard regression analysis was used to evaluate the independent association between patient, disease and treatment characteristics and length of hospital stay. Independent variables with a significant association in univariate analyses were included in multivariable analyses.

Proportional-odds model

To evaluate the influence of our study variables on severity of complications according to the Clavien-dindo score, ordinal logistic regression analysis was performed. Univariate ordinal logistic regression analyses was used to determine the association between our primary study variables and an increase of the Clavien-dindo score. All study variables were evaluated for goodness of fit, using a test of parallel lines. All significant variables were subsequently evaluated using an ordinal logistic regression analysis, thus creating a proportional-odds model.

All statistical analyses were performed by using SPSS version 20.0.

RESULTS

A total of 129 patients were enrolled in the study, of whom 59% were male. The median age was 67 years, 47% of patients were seventy years and older. The majority of patients suffered from malignant disease (67%). Eleven percent of patients had an ASA-classification of three or higher. Twenty percent had a MUST score of 2 or higher. Sixty-one percent had an NRS-2002 score of 3 or higher. Sixty percent underwent open surgery and 40% underwent laparoscopic surgery, of which 21% (N=9) converted to an open procedure. Peri-operative anemia was observed in 87% of patients and 23% received packed cells peri-operatively. Twenty-two percent of patients had a Charlson index of 3 or higher. Sixty-two percent had a BMI higher than 25 kg/m^2, the median BMI being 26 kg/m^2.

Two patients died during our study period, both of whom due to post-operative complications. Seventy-five complications were recorded in 52 patients (table 2). Post-operative complications occurred more often among patients with peri-operative transfusion (p=0.000), MUST ≥2 (p=0.001), ASA ≥3 (p=0.004), and age ≥70 (p=0.013) (table 3). The NRS-2002 was not significantly associated with post-operative complications and was therefore not included in our multivariate analyses.

Table 2. Registered complications

Type of complication	**Frequency n (%)**
Post-operative ileus	20 (27)
Anastomotic leakage	8 (11)
Wound infection opened at bedside	7 (9)
Presacral abcess	6 (8)
Pneumonia	6 (8)
Abdominal sepsis	6 (8)
Urinary tract infections	4 (6)
Abdominal abcess	4 (6)
Line infections	3 (4)
Ostomy problems requiring re-operation	2 (3)
Death	2 (3)
TIA/CVA	1 (1)
Atrial fibrillation	1 (1)
Myocardial infarction	1 (1)
Pleural fluid requiring chest tube	1 (1)
hypotension	1 (1)
Deep Vein Thrombosis	1 (1)
Bleeding requiring re-operation	1 (1)
Total	**75**

Table 3. Univariate analyses of all study parameters on post-operative complications

Study parameters		n (%) patients without complications (n 82)	n (%) patients with complications (Grade≥2) (n 47)	*P*
Perioperative transfusion				0.000
	no	72 (72%)	28 (28%)	
	yes	10 (35%)	19 (65%)	
MUST				0.001
	<2	73 (71%)	30 (29%)	
	≥2	9 (35%)	17 (65%)	
ASA				0.004
	<3	78 (68%)	37 (32%)	
	≥3	4 (29%)	10 (71%)	
Age				0.013
	<70	50 (74%)	18 (26%)	
	≥70	32 (53%)	29 (47%)	
Anemia[1]				0.065
	no	14 (82%)	3 (18%)	
	yes	67 (60%)	44 (40%)	
Procedure				0.151
	lap	33 (72%)	13 (28%)	
	open	49 (59%)	34 (41%)	
Alcohol[2]				0.167
	≤2	36 (59%)	25 (41%)	
	>2	46 (71%)	19 (29%)	
BMI				0.235
	<30	28 (57%)	21 (43%)	
	≥30	54 (68%)	26 (32%)	
NRS-2002				0.227
	<3	35 (70%)	15 (30%)	
	≥3	47 (60%)	32 (40%)	
Sex				0.579
	male	50 (66%)	26 (34%)	
	female	32 (60%)	21 (40%)	
Charlson				0.723
	<3	65 (64%)	36 (36%)	
	≥3	17 (61%)	11 (39%)	
Disease				0.796
	benign	28 (65%)	15 (35%)	
	malignant	54 (63%)	32 (37%)	

Univariate analysis for each study variable with corresponding P-value for the occurrence of postoperative complications (Clavien-dindo ≥ 2)
*1: one missing value
*2: Three missing values

Multiple logistic regression analysis of these variables showed that both peri-operative transfusion (OR 2.78; 95% CI 1.05-7.40) and MUST ≥2 (OR 2.87; 95% CI 1.05-7.87) remained significant independent predictors for the occurrence of post-operative complications (table 4). In the pre-operative risk stratification, both MUST (OR 3.82; 95% CI 1.48-9.88) and ASA (OR 3.31; 95% CI 0.89-12.25) remained significant independent predictors for the occurrence of complications whereas age did not (Table 4).

Table 4 Multivariate model for post-operative complications

	Pre-operative risk stratification		**Peri-operative risk stratification**		**Proportional Odds Model**	
	Odds ratio (95% CI)	***P***	**Odds ratio (95% CI)**	***P***	**Odds ratio 95% CI)**	***P***
Peri-operative transfusion	Not included		2.78 (1.05-7.40)	0.04	2.59 (2.14-6.20)	0.03
MUST ≥2	3.82 (1.48-9.88)	0.006	2.87 (1.05-7.87)	0.04	2.78 (1.13-6.79)	0.03
ASArisk ≥3	3.31 (0.89-12.25)	0.07	2.63 (0.68-10.17)	0.16	2.85 (0.95-8.57)	0.06
Agerisk ≥70	1.90 (0.86-4.22)	0.11	1.77 (0.78-4.00)	0.17	1.94 (0.43-4.10)	0.08
Procedure *[1]	Not included		Not included		1.56 (0.72-3.41)	0.26

Multivariate analysis of study variables shown to be significant in univariate analysis. Left column: multivariate analysis for risk factors that could be ascertained preoperatively i.e. preoperative risk stratification. Middle column: changes in the multivariate analysis when a perioperative risk factor (perioperative transfusion) is added to multivariate model i.e. perioperative risk stratification. Right column: proportional odds model, this model shows proportional odds of each study variable on the increase of complications severity, i.e. the odds of an increase in Clavien-dindo score.
*1: open versus laparoscopic surgery

Our proportional odds model showed that peri-operative transfusion (OR 2.59; 95% CI 2.14-6.20) and MUST ≥2 (OR 2.78; 95% CI 1.13-6.79) were significant independent risk predictors for a progression of the Clavien-Dindo complication grade, and that ASA ≥3 (OR 2.85; 95% CI 0.95-8.57) and age ≥70 (OR 1.94; 95% CI 0.43-4.10) approached significant levels (table 4).

Peri-operative transfusion (p=0.000), age ≥70 (p=0.001), open surgery (p=0.004), MUST ≥2 (p=0.013), ASA ≥3 (p=0.023) and anemia (p=0.027) were significantly associated with increased length of hospital stay. (table 5)

Cox-regression analysis showed that peri-operative transfusion (HR 1.64; 95% CI 1.15-2.35), age ≥70 (HR 1.50; 95% CI 1.05-2.16) and open surgery versus laparoscopic surgery (HR 1.39; 95% CI 0.94-2.05) were independent predictors for increased length of hospital stay, whereas both ASA ≥3 and MUST ≥2 were not. Results of the pre-operative stratification showed that age ≥70 (HR 1.64; 95% CI 1.15-2.35) and open surgery versus laparoscopic surgery (HR 1.48; 95% CI 1.00-2.19) were independent predictors for increased length of hospital stay. (table 6)

Table 5. Univariate analyses of all study parameters on length of hospital stay

Study parameters		n (%)	median length of hospital stay (days)	P (log rank)
Perioperative transfusion				0.000
	no	100 (78%)	7,0	
	yes	29 (22%)	17,0	
Age				0.001
	<70	68 (53%)	6,0	
	≥70	61 (47%)	10,0	
Procedure				0.004
	laparoscopic	64 (36%)	6,0	
	open	83 (64%)	8,0	
MUST				0.013
	<2	103 (82%)	7,0	
	≥2	26 (18%)	11,0	
ASA				0.023
	<3	115 (89%)	7,0	
	≥3	14 (11%)	13,0	
Anemia[1]				0.027
	No	17 (13%)	6,0	
	yes	111 (87%)	8,0	
BMI				0.053
	<25	49 (38%)	8,0	
	≥25	80 (62%)	7,0	
NRS-2002				0.078
	<3	50 (39%)	7,0	
	≥3	79 (61%)	8,0	
Disease				0.122
	benign	43 (33%)	6,0	
	malignant	86 (67%)	8,0	
Sex				0.394
	male	76 (59%)	8,0	
	female	53 (41%)	7,0	
Charlson				0.850
	<3	101 (78%)	7,0	
	≥3	28 (22%)	8,0	
Alcohol[2]				0.735
	≤2	61 (47%)	8,0	
	>2	65 (53%)	7,0	

Univariate analysis for each study variable with corresponding P-value for median length of hospital stay
*1: one missing value
*2: Three missing values

Table 6. Multivariate model for length of hospital stay

	Pre-operative risk stratification		Peri-operative risk stratification	
	Hazard ratio (95%-CI)	***P***	**Hazard ratio (95%-CI)**	***P***
Peri-operative transfusion	Not included		2.40 (1.45-4.00)	0.001
Age risk ≥70	1.64 (1.15-2.35)	0.007	1.50 (1.05-2.16)	0.03
Open surgery (vs laparoscopic)	1.48 (1.00-2.19)	0.048	1.39 (0.94-2.05)	0.10
ASA risk ≥3	1.57 (0.87-2.84)	0.14	1.17 (0.63-2.17)	0.61
MUST ≥2	1.24 (0.81-2.08)	0.29	1.05 (0.65-1.71)	0.84

Multivariate analysis of study variables shown to be significant in univariate analysis. Left column: multivariate analysis of study variables shown to be significant in univariate analysis. Right column: changes in the multivariate analysis when a perioperative risk factor (perioperative transfusion) is added to multivariate model i.e. perioperative risk stratification.

DISCUSSION

The goal of this study was to assess whether pre-operative nutritional risk can be regarded as an independent predictor for post-operative complications and increased length of hospital stay. Our data shows that nutritional risk as measured by the MUST (grade ≥2), is an independent predictor for the occurrence of post-operative complications, but not for increased length of hospital stay. Furthermore, ordinal regression analyses suggest that MUST is an independent predictor for increased complication severity, as measured by the Clavien-Dindo score.

Kyle et al. previously tested the sensitivity and specificity for both the MUST and NRS-2002 with respect to nutritional risk [4]. That study concluded that both screening tools showed comparable predictive power, and that the NRS-2002 had a higher sensitivity and specificity than the MUST for the prediction of nutritional risk. Tangvik et al, investigated the association between nutritional status and clinical outcomes in 3279 patients [13]. Their study concluded that each of the four initial screening questions of the NRS-2002 identified nutritional risk and were strong predictors of hospitalisation, morbidity and mortality among hospitalised patient. Tangvik also points out that using the full survey did not result in any improvement in the prediction of adverse outcomes. Kuppinger et al studied the relative importance of nutritional risk screening along with established medical, anaesthetic and surgical predictors of postoperative morbidity and mortality in 653 patients by evaluating different components of the NRS-2002 separately [11]. Their study suggest that in abdominal surgery, preoperative investigation of feeding habits, i.e. the question of reduction of intake in the four weeks prior to surgery, is the most significant component of the NRS-2002. Ho et al, prospectively investigated the correlation between gastrointestinal operations and short term outcomes in 943 patients by using a Chinese version of the MUST (C-MUST) [13]. The only difference of the C- MUST with the MUST is the lower cutoff values for BMI score. Their study showed that preoperative malnutrition as measured with the C-MUST was an important predictor of poor clinical outcomes in

Hong Kong patients undergoing gastrointestinal surgical procedures. Considering the above-mentioned works of Tangvik and Kuppinger, together with the findings of this study, we question the applicability of the NRS-2002 for the detection of nutritional risk for postoperative morbidity on an ageing population undergoing major abdominal surgery. A possible explanation for the inconsistency between the NRS-2002 and MUST is that the NRS-2002 scores major abdominal surgery as two points and age of seventy years or higher as one point. Considering the fact that all patients undergoing gastro-intestinal surgery score as undergoing major abdominal surgery and the mean age of our cohort was 67 years, this may explain the high estimation of risk for patients in our cohort. Therefore, the shorter, easier to use, MUST is suggested to be the preferred tool to detect preoperative nutritional risk for postoperative morbidity in this cohort.

The studies of Kruizinga et al, Tangvik et al and Ho et al all showed nutritional risk to be associated with an increased length of hospital stay [7, 13-15]. Kruizinga also extrapolated that early nutritional intervention has been shown to reduce length of hospital stay [14]. The incremental cost of a 1-day reduction in the length of hospital stay in a malnourished patient group, through extra nutritional care and dietetic treatment has been shown to be €76 (US$91.2). If the mean costs of a 1-day stay in the hospital are €476 (US$571.2) for university hospitals and €337 (US$404.4) for general hospitals, significant cost benefit can be obtained by the early detection and treatment of undernourished patients [14]. The fact that MUST was not associated with an increased length of hospital stay in our multivariate analysis, could possibly be explained by the fact that in our cohort only few high-grade complications (grade 4:ICU admission and grade 5:death, leading to hospitalization) occurred. This could have lead to the MUST being significant in our multivariate model for the occurrence of postoperative complications but not for length of hospital stay. Another possible explanation could be that our study investigated the time to discharge, not the time upon patients were ready for discharge. This mean that social reasons for delayed discharge were not taken into consideration.

A disadvantage to the prospective nature of this study is the fact that inclusions over the available study period were limited, leading to limited statistical power. Another possible limitation is that both the MUST and NRS-2002 screening were performed by only one researcher. Therefore, inter-observer reliability could not be ascertained.

It must be noted that our binary evaluation of complications did not include grade one complications due the assumption that grade one complications have little effect on post-operative morbidity and length of hospital stay. For our proportional odds model we selectively included grade one complications, only to including wound infections opened at bedside. Due to a limited cohort it was not possible to perform a cox proportional hazard regression using all suffered complications per patient. We opted to use the complication that had the greatest impact on the patient, i.e the highest Clavien-Dindo score. In our study we included four patients who received nutritional support in the form of total parenteral nutrition. These patients were screened upon admission to hospital. It can be

assumed that these patients had a better nutritional status at the time of operation than measured upon admission. This could have resulted in an overestimation of nutritional risk in these patients and consequently an underestimation of its effect on post-operative complications.

Our study suggests that nutritional risk screening, in the form of the MUST as well as ASA ≥3 are both independent predictors for post-operative complications. Moreover, our data suggests the MUST to be an independent predictor for increased complication severity, as measured by the Clavien-Dindo score. However, risk for malnutrition did not significantly predict increased length of hospital stay.

CONCLUSION

Malnutrition is a common condition, which can be adequately detected using relatively simple screening tools as the MUST. Our study confirms that an impaired nutritional status leads to an increased post-operative morbidity. This emphasizes the need for an adequate implementation of screening for malnutrition before major gastro-intestinal surgery.

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CHAPTER

Value of sarcopenia assessed by computed tomography for the prediction of postoperative morbidity following oncological colorectal resection: A comparison with the malnutrition screening tool

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ABSTRACT

Background: Computed tomography (CT) can be used for accurate estimation of whole-body muscle mass and muscle density and for detection of sarcopenia. The goal of this study was to evaluate the additional value of CT measured sarcopenia and muscle attenuation alongside the Malnutrition Universal Screening Tool (MUST) for the prediction of post-operative morbidity after oncological colorectal resection, whilst correcting for known risk factors.

Methods: A prospective cohort study of 80 patients undergoing oncological colorectal resection. Patients were screened for nutritional risk upon admission using the MUST. Additionally, preoperative CT scans were used to determine skeletal muscle mass for the detection of sarcopenia and skeletal muscle radiation attenuation. Univariate and multivariable analyses were performed to evaluate associations between the MUST, muscle attenuation and sarcopenia and post-operative complications measured by the Clavien-Dindo score.

Results: American Society of Anesthesiology-classification (ASA) ≥3, age ≥70, MUST ≥2 were significantly associated with a higher risk for postoperative complications (Clavien-Dindo score ≥2) in univariate analysis ($p<0.05$). Multivariate analyses showed that only MUST ≥2 remained significantly associated with postoperative complications when corrected for age ($p=0.03$, OR 5.8, 95%CI 1.1-29.6), but not when corrected for age ≥70 and ASA ≥3. Muscle attenuation and sarcopenia were not significantly associated with postoperative complications.

Conclusion: Our results suggest that using CT measured sarcopenia may not have additional value over the MUST for the prediction of increased short-term post-operative morbidity after oncological colorectal resection. It also underlines the importance of currently implemented easy-to-use nutritional screening tools (MUST).

INTRODUCTION AND RATIONALE

In the Netherlands, colorectal cancer (CRC) constitutes the second most common type of malignant disease. According to statistics from the population-based Netherlands Cancer Registry almost 15,600 new cases of CRC were diagnosed in the Netherlands in 2015 (almost 8,900 men and almost 6,700 women) [1]. A general epidemiological trend in CRC shows an increase in incidence over time, increased incidence with age and decreasing mortality [1].

Nutritional status has been shown to be an independent risk factor for post-operative complications amongst all patients undergoing colorectal resection [2-5]. The European Society for Nutrition and Metabolism (ESPEN) recommends the use of the Malnutrition Universal Screening Tool (MUST) for clinical use [6]. The use of computed tomography (CT) has been suggested to be a reliable tool for accurate estimation of whole-body muscle mass and the detection of sarcopenia [7-14]. This technique uses CT images at the level of the third lumbar vertebra (L3) to determine cross-sectional area of skeletal muscle at L3, which when corrected for the patient's height is a measure of lean body mass [7-18]. Besides estimation of body mass, e.g. for the detection of sarcopenia, CT image analysis has been used for the estimation of muscle density, also known as skeletal muscle radiation attenuation (SM-RA). SM-RA measures the mean value of Hounsfield units in skeletal muscle mass at the L3 level, and may be used for the estimation muscle density, in addition to muscle mass [19]. Besides the estimation of mass, e.g. for the detection of sarcopenia, CT image analysis can be used for the estimation of muscle density, also known as skeletal muscle radiation attenuation (SM-RA). SM-RA measures the mean value of Hounsfield units in skeletal muscle mass and is considered to be a radiological marker indicative of myosteatosis [19-22]. Myosteatosis is characterized by increased inter- and intra-myocellular fat stores, which can be influenced by dietary pattern and activity changes. It has also been shown to be related to reduced physical fitness and reduced muscle function [19, 23-26]. The goal of this study was to assess whether CT measured sarcopenia and or myosteatosis has additional value besides the MUST for the prediction of postoperative complications after oncological colorectal resection.

STUDY DESIGN & METHODS

A prospective cohort study of patients undergoing elective surgical intervention was conducted. All patients aged 18 years and older undergoing elective surgery to the gastro-intestinal tract between October 2012 and July 2013 in VieCuri teaching hospital in the south of the Netherlands were included (N=80). Patients with American Society of Anesthesiology (ASA)-classification V, severe liver cirrhosis – Child grade C, end stage renal disease requiring dialysis, severe heart disease – New York Heart Association class IV and chronic obstructive pulmonary disease (COPD) requiring (home)oxygen therapy or

an interval greater than 2 months between the time of the scan and the operation were excluded (N=6).

Prior to this study, a nutritional screening and intervention algorithm had successfully been implemented at our hospital in accordance with ESPEN guidelines. All patients were screened using the MUST screening tool upon hospital admission. Patients with MUST scores of 2 or higher received a nutritional intervention in the form of additional high caloric food products before surgery, in accordance with ESPEN guidelines. As a nutritional screening and treatment algorithm was already in place, no change in the nutritional intervention regime was implemented in this study.

Besides nutritional status, known risk factors for post-operative complications were recorded: age (<70 and ≥70 years), ASA-classification (<3 and ≥3), planned type of procedure (laparoscopic or open: conversion from laparoscopic to open surgery was classified as open surgery) and peri-operative blood transfusion. Comorbid diseases were categorized according to the Charlson Comorbidity Index. In the Charlson Comorbidity index, a weighted score is assigned to each of 17 comorbidities and the sum of the index score is an indicator of disease burden and an estimator of mortality. For the analyses we classified the Charlson index as a binary variable; a score of 3 or more was considered an increased comorbidity risk [27].

The primary endpoint was the occurrence of post-operative complications within 30 days after surgery, which were prospectively registered. Post-operative complications were categorized by severity as proposed by Clavien-Dindo [28]. According to this classification, complications are defined as any deviation from the normal post-operative course. Complications were graded from I to V, based on the extent of intervention needed to correct the complication. In the case of several complications these were recorded separately, and the most severe complication was used in the statistical analyses. For the statistical analyses, we classified postoperative complications as a binary outcome: complications graded 2 or higher were categorized as the occurrence of serious post-operative complications, whereas grade 0 and 1 were graded as no serious complications. Data from this cohort has previously been used for a different study [29].

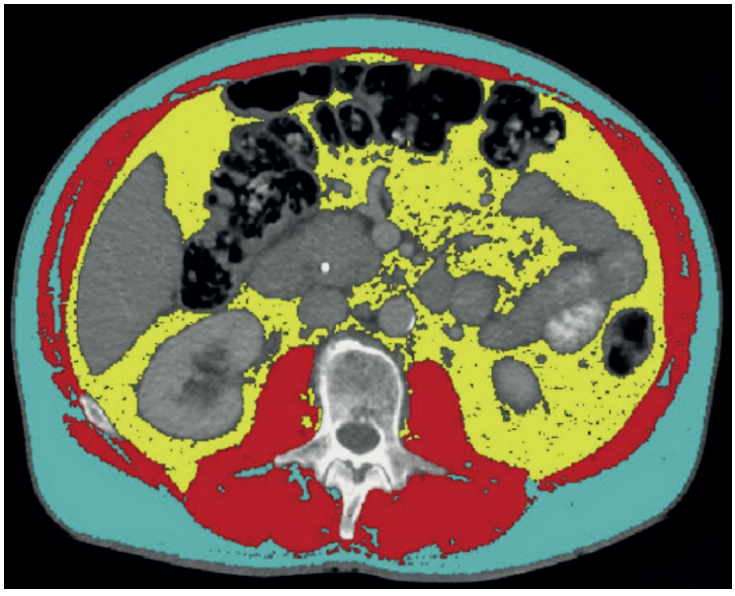
CT measured sarcopenia

Pre-operative abdominal CT scans were available for all patients (N=80). Exclusion was on the basis of poor quality of CT scans (N=5), scans not showing the abdominal wall (N=4), absence of the third lumber vertebra on the scan (N=2), and/or an interval greater than 2 months between the time of the scan and the operation (N=6).

Muscle mass was measured by analyses of electronically stored CT images, which had been routinely taken for diagnostic purposes. The third lumbar vertebra (L3) was used as a standard landmark to measure muscle cross-sectional area in cm^2. Skeletal muscle tissue was identified and quantified on CT images by means of Hounsfield unit (HU) thresholds (−29 to +150 HU). The total cross-sectional area of skeletal muscles at L3 was computed by

use of Slice-O-matic software, version 5.0 (Tomovision, Montreal, QC, Canada) (figure 1). Total muscle area at L3 normalized for body height (L3 skeletal muscle index, cm^2/m^2) is linearly related to whole-body muscle mass. Based on the L3 skeletal muscle index, sarcopenia was defined using sex-specific cutpoints: 43 cm^2/m^2 for males with BMI < 25.0 kg/m^2 and 53 cm^2/m^2 for males with BMI ≥25.0 kg/m^2; the cutpoint for sarcopenia in females was 41 cm^2/m^2, irrespective of BMI [30, 31]. Besides muscle mass, muscle radiation attenuation (i.e. the mean HU of muscle tissue) was also determined as an indicator of muscle density. Study participants were divided in groups of high versus low muscle attenuation based on the median value as cutoff (low: <34.1 HU; high: ≥34.1 HU).

Figure 1. Segmented CT image at the 1.3 level showing subcutaneous fat in blue, muscle in red, and visceral fat in yellow.



Statistical Analyses

Frequencies are presented as absolute numbers and percentages. Univariate analyses were used to determine the association between each individual variable and the outcome variable using Chi-square analysis or Fisher's Exact test in case of low expected frequencies.

Multivariable analyses

Confounding between sex, age (<70 and ≥70), ASA-classification (<3 and ≥3) and MUST and CT-measured sarcopenia was tested by consecutively evaluating them in our multivariate model. Multiple logistic regression analysis was used to analyse the independent association between our primary study variables and the occurrence of post-operative

complications (yes versus no). Only variables with a significant association in univariate analyses were included in multivariable analyses. Due to limited statistical power allowing for a limited number of variables to be included in our multivariable model, we opted to run several multivariable models, i.e., an uncorrected model containing the primary study variables and four models correcting for other risk factors. The first model only included the two main variables of our study (MUST and sarcopenia). The second contained muscle attenuation instead of sarcopenia. In the third model, age (age ≥70) was added. The fourth and final model contained both the primary study variables with the addition of age and ASA ≥3. All statistical analyses were performed by using SPSS version 20.0.

RESULTS

Of the 80 included patients, a total of 63 were selected for CT analysis, of whom 64% were male. The mean age was 69 years (SD 10.5) and 48% of patients were aged seventy years or older. Fifty-two percent of patients were sarcopenic before surgery (n=33). Five percent had a BMI higher than 30 kg/m^2 (N=3), of which one was sarcopenic. The mean BMI was 26 kg/m^2. Sixteen percent had a MUST score of 2 or higher (N=10). Eleven percent of patients had an ASA-classification of three or higher (N=7). Forty-four percent (N=26) underwent open surgery and 56% (N=27) underwent laparoscopic surgery, of which 9% (N=3) converted to an open procedure. Twenty-five percent (N=16) received packed cells peri-operatively. Twenty-one percent of patients (N=16) had a Charlson index of 3 or higher. Sarcopenic patients had higher MUST scores, however this was not significant (p=0.06). (Table 1). Three percent of patients (N=2) died due to post-operative complications, one of which was sarcopenic before surgery. Thirty-eight percent of patients (N=24) suffered complications of which the highest-grade complication was used for analyses (4x grade 1, 7x grade 2, 8x grade 3, 2x grade 4, 2x grade 5) (Table 2).

Univariate analysis showed that ASA ≥3, age ≥70 and MUST ≥2 were significantly associated with a higher risk for postoperative complications (Clavien-Dindo score ≥2) (p<0.05) and lower than median muscle attenuation approached significant association (p=0.05) (Table 3). Multivariable analyses showed that MUST ≥2 (p=0.03, OR 5.8, 95%CI 1.1-29.6) remained significantly associated with the occurrence of postoperative complications after adjustment for differences in age. After additional adjustment for ASA, only age ≥70 (p=0.05, OR 3.4, 95%CI 1.0-11.5) remained significant, whilst ASA ≥3 approached significant levels (p=0.08, OR 8.5, 95%CI 0.8-94.5). MUST ≥2 (p=0.12, OR 4.1, 95%CI 0.7-24.7) and muscle attenuation (p=0.18, OR 0.5, 95%CI 0.1-1.4) were not significant in multivariable analysis. All multivariable models showed sarcopenia (p≥0.66) to be non-significant (Table 4).

Table 1. Division of study variables in sarcopenic and non-sarcopenic groups

Study parameters	Sarcopenia no (*n*=30)	Sarcopenia yes (*n*=33)	*P*-value
Muscle Attenuation (HU)			0.00
<median	7 (23%)	24 (77%)	
≥median	23 (72%)	9 (28%)	
Muscle area (CM²)			0.00
<median	8 (24%)	25 (66%)	
≥median	22 (73%)	8 (27%)	
MUST			0.06
<2	28 (53%)	25 (47%)	
≥2	2 (20%)	8 (80%)	
Perioperative transfusion			0.13
no	25 (53%)	22 (47%)	
yes	5 (31%)	11 (69%)	
Age groups			0.25
<70	18 (55%)	15 (45%)	
≥70	12 (40%)	18 (60%)	
ASA			0.43
<3	28 (50%)	28 (50%)	
≥3	2 (29%)	5 (71%)	
Charlson			0.46
<3	25 (50%)	25 (50%)	
≥3	5 (39%)	8 (61%)	
BMI groups			0.60
<30	28 (47%)	32 (53%)	
≥30	2 (67%)	1 (33%)	
Sex			0.82
male	19 (49%)	20 (51%)	
female	11 (46%)	13 (54%)	
Procedure			0.84
laparoscopic	18 (49%)	19 (51%)	
open	12 (46%)	14 (54%)	
Tumor Stage			0.97
Stage 1	9 (50%)	9 (50%)	
Stage 2	6 (46%)	7 (54%)	
Stage 3	10 (50%)	10 (50%)	
Stage 4	5 (42%)	7 (58%)	

Division of study variables in sarcopenic and non-sarcopenic groups. P-value calculated by Chi-square or Fisher Exact.
Muscle CM² was defined as lower or higher than the median (men: <154.5 cm²/m² and ≥154.5 cm²/m² woman <103.3 cm²/m² and ≥103.3 cm²/m²), Muscle attenuation was defined as higher or lower than the media value (<34.1HU or ≥34.1HU).

Table 2. Registered complications

Type of complication	Frequency *n*	Clavien-Dindo-Score
Anastomotic leakage	5	3b
Wound infection opened at bedside	5	1
Abdominal sepsis	4	4a/b
Post-operative ileus	3	2
Pneumonia	3	2
Presacral abcess	2	3a
Urinary tract infections	2	2
Abdominal abcess	2	3a
Line infections	2	2
Ostomy problems requiring re-operation	2	3b
TIA/CVA	1	4
Deep Vein Thrombosis	1	2
Bleeding requiring re-operation	1	3b
Death	2	5
Total	**35**	

Total number of registered complications Clavien-Dindo ≥2.

Table 3. Univariate analyses of all study parameters on post-operative complications clavien-dindo score ≥2

ASA	*Complications n (%)*		*P-Value*
	No (n=42)	*Yes (n=21)*	<0.01
<3	41 (73%)	15 (27%)	
≥3	1 (14%)	6 (86%)	
Age			<0.01
<70	27 (82%)	6 (18%)	
≥70	15 (50%)	15 (50%)	
MUST			0.01
<2	39 (74%)	14 (26%)	
≥2	3 (30%)	7 (70%)	
Muscle Attenuation			0.05
<median	17 (55%)	14 (45%)	
≥median	25 (78%)	7 (22%)	
Perioperative transfusion			0.10
no	34 (73%)	13 (17%)	
yes	8 (50%)	8 (50%)	
Procedure			0.20
lap	27 (73%)	10 (27%)	
open	15 (56%)	11 (44%)	
BMI groups			0.25
<30	41 (68%)	19 (32%)	

≥30	1 (33%)	2 (67%)	
Sex			0.27
male	28 (70%)	12 (30%)	
female	14 (61%)	9 (39%)	
Muscle CM²			0.28
<median	24 (73%)	9 (26%)	
≥median	18 (60%)	12 (40%)	
Sarcopenia			0.59
no	21 (70%)	9 (30%)	
yes	21 (64%)	12 (36%)	
Charlson			0.66
<3	34 (68%)	16 (32%)	
≥3	8 (61%)	5 (39%)	

Univariate analysis for each study variable with corresponding P-values for the occurrence of postoperative complications (Clavien-dindo ≥ 2), calculated by Chi-square or Fisher Exact.
Muscle CM² was defined as lower or higher than the median (men: <154.5 cm^2/m^2 and ≥154.5 cm^2/m^2 woman <103.3 cm^2/m^2 and ≥103.3 cm^2/m^2), Muscle attenuation was defined as higher or lower than the media value (<34.1HU or ≥34.1HU).

Table 4. Multivariate analyses of all study parameters on post-operative complications clavien-dindo score ≥2

Uncorrected	Alternate	Age corrected	Age +ASA corrected
MUST ≥2 (P=0.02 OR 6.6 95%CI 1.4-30.9)	*MUST ≥2 (P=0.04 OR 5.1 95%CI 1.1-23.6)*	*Age≥70 (P=0.02 OR 4.0 95%CI 1.2-13.3)*	*Age ≥70 (P=0.05 OR 3.4 95%CI 1.0-11.5)*
Sarcopenia (P=0.91 OR 0.9 95%CI 0.3-2.9)	*Muscle Attenuation Index (P=0.18 OR 0.5 95%CI 0.1-1.4)*	*MUST ≥2 (P=0.03 OR 5.8 95%CI 1.1-29.6)*	*ASA ≥3 (P=0.08 OR 8.5 95%CI 0.8-94.5)*
		Sarcopenia (P=0.70 OR 0.8 95%CI 0.2-2.7)	*MUST ≥2 (P=0.12 OR 4.1 95%CI 0.7-24.7)*
			Sarcopenia (P=0.66 OR 0.8 95%CI 0.2-2.7)

Multivariate analysis for each study variable significant in univariate analyses, calculated by multiple binary logistic analyses. Left to right. First; uncorrected model containing MUST and Sarcopenia. Second Alternative model containing muscle attenuation instead of Sarcopenia. Third; model corrected for Age≥70. Fourth; model corrected for both Age≥70 and ASA≥3.
Muscle attenuation was defined as higher or lower than the media value (<34.1HU or ≥34.1HU).

DISCUSSION

Our study shows a high prevalence of sarcopenia in patients undergoing oncological colorectal resection (52%) but failed to show a significant association with post-operative complications. The MUST≥2 and lower muscle attenuation were univariately associated with postoperative complications. However, when including both measures

in multivariable analyses, both failed to show a significant association with postoperative complications.

Malnutrition as measured with a variety of validated malnutrition screening tools has shown to be a predictor for worse outcome after colorectal, gastric, and general abdominal surgery [2-5, 29]. More recently, body composition measurement through CT image analysis has become increasingly common. Several studies previously showed a relationship between CT-measured sarcopenia and increased post-operative morbidity and mortality following colorectal resection [8-10, 18]. Tegels et al. found a high prevalence of sarcopenia in gastric cancer patients, but no association with worse outcome [14]. In addition, Lodewick et al. found that obesity and sarcopenic obesity did not worsen disease-free survival, overall survival or complication rates after partial liver resection for colorectal liver metastasis [17]. Muscle attenuation may be independently prognostic of survival in patients with solid based tumors [31]. This could possibly imply that muscle quality, i.e. muscle attenuation, may be of importance besides muscle quantity measurement, i.e. muscle volume measurements. However, in our small cohort, muscle attenuation did not reach statistical significance in predicting postoperative complications in addition to MUST. This might have been due to the small number of patients in the current study.

The clinical implementation of CT sarcopenia measurement is time-consuming and therefore costly. Our study used pre-operative CT scans that were performed for oncological dissemination purposes and not for the purpose of sarcopenia measurement. This leads to a substantial percentage of CT scans being unusable for sarcopenia measurements (21%) and therefor-limited statistical power. A strength of this study is that both the MUST screening and CT measurements were performed by only one researcher, reducing the influence of potential inter-observer differences, however inter- and intra-observer variances could not be established. It must be noted that ASA ≥3 has an excessively wide 95% confidence interval in our multivariate model (OR 8.5, 95%CI 0.8-94.5). This is most likely due to the fact that this variable shows a substantial skewness with only one of 63 cases having an ASA ≥3 without complications. This combined with a limited power consequent to the low number of outcome events causes this variable to have a profound impact on our multivariate models.

Our results suggest that CT-measured sarcopenia does not hold additional value over the MUST for the prediction of increased short-term post-operative morbidity after oncological gastrointestinal and colorectal surgery. It also underlines the importance of currently implemented easy-to-use nutritional screening tools (MUST).

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CHAPTER

Myosteatosis to predict inferior perioperative outcome in patients undergoing orthotopic liver transplantation

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ABSTRACT

Background: Muscle wasting, and alterations of body composition (BC) are linked to clinical outcomes in numerous medical conditions. The role of myosteatosis in post-transplant outcomes remains to be determined. Here we investigated skeletal muscle mass and myosteatosis as prognostic factors in patients undergoing orthotopic liver transplantation (OLT).

Materials and methods: The data of 225 consecutive OLT-recipients from a prospective database were retrospectively analysed (05/2010-12/2017). Computed-tomography-based skeletal-muscle-index/SMI (muscle mass), visceral-fat-area/VFA (visceral adiposity) and mean skeletal-muscle-radiation-attenuation/SM-RA (myosteatosis) were calculated using a segmentation tool. Cut-off values of myosteatosis resulted in a good stratification of patients into low- and high-risk groups in terms of morbidity (Clavien-Dindo≥3b).

Results: Patients with myosteatosis had significantly higher complication-rates (90-days CCI 68±32 vs. 44±30, p<0.001) and also displayed significantly longer intensive-care (18±25 vs. 11±21 days, p<0.001) and hospital stay (56±55 vs. 33±24 days, p<0.001). Estimated costs were 44% higher compared to patients without myosteatosis. Multivariable analysis identified myosteatosis as an independent prognostic factor for major morbidity (OR:2.772, CI:1.516-5.066, p=0.001). Adding myosteatosis to the well-established Balance-of-Risk-(BAR)-score resulted in an increased prognostic value compared to the original BAR-score.

Conclusion: Myosteatosis may be a useful parameter to predict perioperative outcome in patients undergoing OLT, supporting the role of muscle quality (myosteatosis) over quantity (muscle mass) in this setting.

INTRODUCTION

Body composition (BC) varies among individuals as a result of differences in age, body density, and the degree of obesity [1]. Muscle depletion is frequently seen in critical ill patients and BC correlates with clinical outcomes in various medical conditions [2, 3]. Besides the pure loss of muscle mass and function known as sarcopenia, qualitative structural changes, such as myosteatosis, characterized by the presence of inter- and intramyocellular fat deposition, have been shown to be associated with reduced muscle strength and poor clinical outcomes in patients undergoing surgery for colorectal or pancreatic cancer [4-7].

In the face of critical organ shortage with increased utilization of marginal allografts procured from extended criteria donors (ECD), one of the most challenging clinical decisions in the liver transplantation setting is the optimal risk-quantification and the identification of high-risk recipients and/or donor-recipient combinations [8]. In this regard, several clinical risk-assessment systems, using donor and recipient factors (e.g. Balance-of-Risk or BAR score), have been developed over the past 10-15 years [8-13]. While some of these models are helpful in predicting post-transplant outcomes, none of these scores include information on the recipient's BC [14]. Cross-sectional computed-tomography (CT) imaging studies are validated and widely available, and as such, BC could be easily integrated in the process of risk-stratification and routine clinical workup of transplant recipients [2, 15].

The importance of nutritional status and its assessment in patients with chronic liver disease is further highlighted by the recent European Association for the Study of the Liver (EASL) and American Association for the Study of Liver Diseases (AASLD) guidelines on nutrition in chronic liver disease [16-18]. Patients with terminal liver disease are frequently burdened by malnutrition and muscle wasting. While some studies have recently reported an association of sarcopenia with clinical outcome in cirrhosis and OLT [15, 19-23], the prognostic value of muscle quality (skeletal muscle density and myosteatosis) compared to muscle quantity (skeletal muscle mass-SMM, as the morphological aspect of sarcopenia) remains to be determined [2, 19-22].

The goal of the present study was to comprehensively assess the role of different CT-based recipient BC profiles and compare the value and limitations of reduced muscle mass and myosteatosis in predicting perioperative outcomes in adult patients undergoing deceased donor OLT.

PATIENTS AND METHODS

Patients and ethics

Between May 2010 and December 2017, all consecutive patients undergoing OLT at the University Hospital RWTH Aachen (UH-RWTH), Aachen, Germany, were considered for

inclusion. Although, during the study period all patients have received a pre-transplant CT imaging, patients with CT scans older than 6 months and/or those not including the L3 level were excluded. Patients receiving living related or split liver transplantation were also excluded. Patients undergoing re-OLT have been assessed related to the primary transplantation and consecutive transplantations were included in the follow-up. The study was conducted at the UH-RWTH in accordance with the current version of the Declaration of Helsinki as well as the Declaration of Istanbul, and the good clinical practice (ICH-GCP) guidelines. The study was approved by the responsible Institutional Review Board of the RWTH-Aachen University (EK 047/18). Informed consent was waived due to the retrospective study design and collection of readily available clinical data.

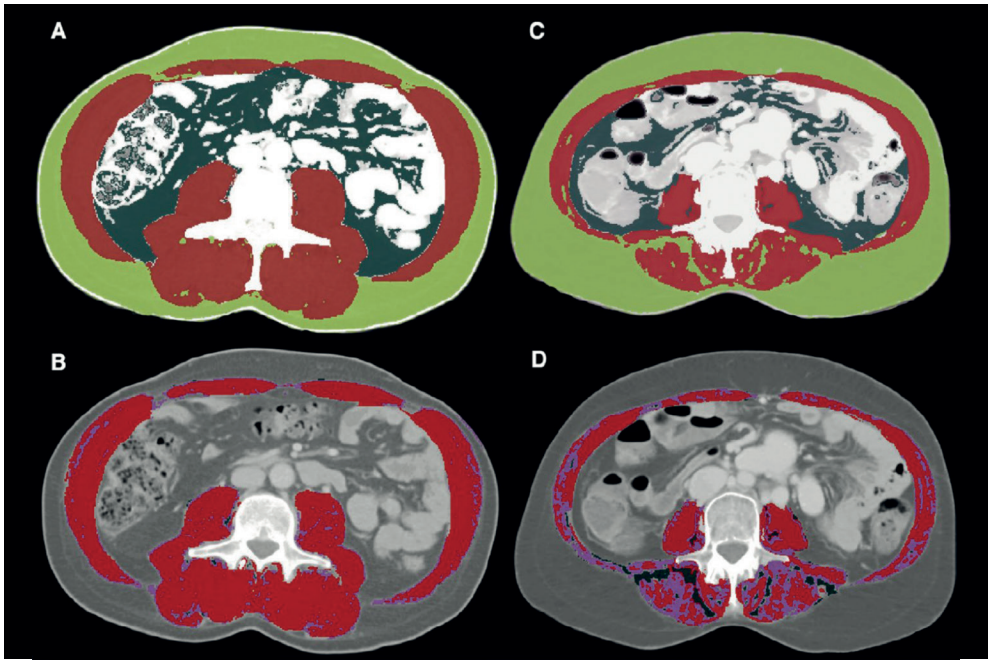
Computed tomography (CT) imaging and segmentation

All CTs were performed by using a Dual-Source-CT-scanner (Siemens Somatom Force, Siemens AG, München, Germany). The technical parameters for CT-imaging were: tube voltage, 120 kVp; 0.5 s/rotation, and 5 mm reconstruction thickness.

Data of the most recent preoperative CT-imaging were retrieved from digital storage in the Picture Archiving and Communication System (PACS). Briefly, a single cross-sectional CT image at the level of the third lumbar vertebra (L3) was used and the segmentation of skeletal muscle and adipose tissue was performed using the 3D Slicer software platform version 4.1 and BC module (https://www.slicer.org/, [24]) in a semiautomatic fashion. Skeletal muscle area, including psoas major, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques, and rectus abdominis were identified and quantified by using attenuation values of -29 to 150 Hounsfield units (HU). Skeletal muscle index (SMI) has been calculated by normalizing the measured muscle area to the square height of the patient (cm^2/m^2). Skeletal muscle radiation attenuation (SM-RA) as an indicator of muscle density and myosteatosis has also been recorded in HUs. Visceral fat area (VFA) was quantified by using the attenuation values -150 to -50 HU. To identify subcutaneous adipose tissue, the attenuation values of -190 to -30 were used (Figure 1). All measurements were performed by the same researcher, experienced in complex image analysis and segmentation techniques.

Based on the SMI and SM-RA, reduced skeletal muscle mass and myosteatosis were defined using cut-off values determined specifically for patients on OLT waiting list (SMI: female 39 cm2/m2, male 50 cm2/m2; SM-RA <41 HU for patients with a BMI up to 24.9 kg/m2 and <33 HU for patients with a BMI≥25 kg/m2) [15, 25]. Established cut-off value of ≥100 cm2 has been used for VFA to identify patients with visceral obesity [15]. Sarcopenic-obesity was defined as the combination of reduced SMI and high VFA. Based on previous literature findings from patient cohorts with terminal liver disease, no differentiation was made based on sex in terms of SM-RA and VFA [15].

Figure 1. Cross sectional computed tomography images of two OLT recipients after segmentation at the level of the third lumbar vertebra



(A and C) Skeletal muscle area (red) was identified by using computed tomography attenuation values of -29 to 150 HU. Subcutaneous fat area (light green) was depicted by using attenuation values of -190 to -30 HU. For visceral fat area (dark green) -150 to -50 HU attenuation values were used. Pictures B and D depict structural alterations of the skeletal muscle on representative images from two OLT recipients. For this, intramuscular adipose tissue has been shown in dark green (-190 to -50 HU), normal attenuation muscle has been marked red (+30 to 150 HU). Myosteatotic, low attenuation muscle was delineated in violet (-29 to 29 HU). Patient 1 (panels A and B) had a normal SMI of 83 cm^2/m^2 and normal muscle structure with low amount of intramuscular adipose tissue and insignificant amount of low attenuation (myosteatotic) muscle (SM-RA: 46 HU). Meanwhile, patient 2 (panels C and D) presented with quantitatively slightly reduced but still normal muscle mass, characterized by an SMI of 62 cm^2/m^2. However, mind the severe qualitative alterations observed in patient 2, with increased amount of intramuscular fat (dark green on panel D) and significant myosteatosis (SM-RA: 32 HU), visualized by the strong presence of low attenuation muscle (violet on panel D).

Data collection and follow-up

Clinical data were obtained from a prospective institutional database and analyzed retrospectively. Various OLT risk-scores (Table 1) have been calculated as described before [10, 13, 26]. Extended criteria donor allografts (ECD) were defined according to the definition of the German Medical Chamber (donor age>65-years, ICU with mechanical ventilation >7 days, BMI >30, histologically confirmed graft steatosis >40%, serum sodium >165 mmol/L, serum alanine and/or aspartate amino-transferase >3x higher than the reference level, serum total bilirubin >2mg/dL) [27]. To assess post-transplant early allograft dysfunction

(EAD) the Olthoff criteria were adopted [28]. Postoperative morbidity was evaluated for all surgical complications observed during the first 90-days following OLT according to the Clavien-Dindo classification (CD) and quantified using the Comprehensive Complication Index (CCI©) [29, 30]. Cost estimation was performed based on recipient age and 90-days CCI score, according to Staigner et al., using a validated online cost-assessment tool for OLT (URL: https://www.assessurgery.com/cost-prediction/) [31]. Recipient pre-OLT performance status has been assessed using the Karnofsky performance score (KPS) [32].

Postoperative transfusions were defined as any blood products given within the first 7-days following OLT. Blood products administered later in the postoperative period were categorized as postoperative complications according to the recommendations of the Clavien-Dindo classification [30]. Length of ICU-stay represents the initial stay after the OLT procedure until the transfer of the patient to our standard care transplantation unit. Hospital stay was defined by the date of admission for OLT and the day of discharge from the UH-RWTH. Readmission to the ICU was included within the total hospital stay. Our transplantation outpatient clinic as well as the responsible general practitioner and/or hepatologist provided all follow-up data used in this study.

Table 1. Donor and recipient characteristics

Characteristics	**n=225**
Donor characteristics	
Donor age (years)	55±16
Donor BMI	30±8
Donor sex ratio (F:M)	108 (48%) : 117 (52%)
DRI[1]	1.77±0.35
Donor cause of death	CVA 138 (61%) Anoxia 51 (23%) Trauma 25 (11%) Other 11 (5%)
ECD[2]	154 (68%)
Recipient characteristics	
Recipient age (years)	54±12
Recipient BMI	27±5
Recipient sex ratio (F:M)	75 (33%) : 150 (67%)
Etiology of liver disease	ALF 31 (14%) HCC 63 (28%) Alcoholic cirrhosis 45 (20%) Viral 15 (7%) PSC/PBC 21 (9%) Graft failure 4 (2%) Other 46 (20%)
Pre-transplant Child-Pugh Score	7±2
Pre-transplant labMELD	20±11
BAR Score[3]	9±6
SOFT Score[4]	15±10
Recipient pre-transplant ICU	56 (25%)
Recipient pre-transplant abdominal surgery	82 (36%)

Recipient pre-transplant encephalopathy	90 (40%)
Karnofsky Performance Score[5]	60±25
SMI (cm^2/m^2) Male:Female	57±39 : 47±11
SM-RA (HU) Male:Female	35±11 : 32±11
VFA (cm^2) Male:Female	130±92 : 94±156
Cold ischemic time (min)	516±139
Warm ischemic time (min)	46±7
Intra-operative platelet transfusions (units)	1±2
Intra-operative red blood cell transfusions (units)	9±8
Intra-operative fresh frozen plasma transfusions (units)	18±10
Post-operative platelet transfusions (units)[6]	1±2
Post-operative red blood cell transfusions (units)[6]	4±6
Post-operative fresh frozen plasma transfusions (units)[6]	6±11

Values were given as mean±standard deviation or numbers and (per cent).
[1]Refers to Feng et al.[9]; [2]Refers to German Medical Chamber Guidelines.[27]; [3]Refers to Schlegel et al.[13]; [4]Refers to Rana et al.[12]; [5]Refers to Kelly et al.[32]; [6]Refers to blood products given during the first 7-days following OLT.
Abbreviations used: POD: postoperative day, BMI: body mass index, DRI: donor risk index, CVA: cerebrovascular accident, ECD: extended criteria donor allografts, ALF: acute liver failure, HCC: hepatocellular carcinoma, PSC: primary sclerosing cholangitis, PBC: primary biliary cirrhosis, AIH: autoimmune hepatitis, MELD: model for end-stage liver disease, PLT: platelet, BAR: balance of risk, SOFT: survival outcomes following liver transplantation, CCI: comprehensive complication index, ICU: intensive care unit, SMI: lumbar 3 skeletal muscle index, SM-RA: lumbar 3 skeletal muscle radiation attenuation, VFA: lumbar 3 visceral fat area.

Surgical- and perioperative approach

All OLT waiting-list indications were discussed and decided within a multidisciplinary liver transplantation board in accordance to the German national and Eurotransplant guidelines. Organ allocation followed national and Eurotransplant regulations. The liver transplantation procedure was performed using a standardized approach of total cava replacement as previously described [33, 34]. The standard perioperative care and immunosuppression regimen consisted of basiliximab, tacrolimus, mycophenolate-mofetil and corticosteroids [33, 34].

Study endpoints and statistical analysis

Ninety-day major morbidity (Clavien-Dindo-CD≥3b) following OLT was chosen as the primary endpoint for the binary logistic regression analyses to demonstrate the association of certain factors with early morbidity [30]. Perioperative outcome, length of ICU- and hospital stay, mortality, EAD, procedural costs comprised the secondary endpoints.

For analysis of categorical data, the Chi-square test and the Fisher's exact test were used. For comparison of continuous variables, the Mann-Whitney U test was applied. In case of continuous data stratified into more than two groups, the one-way ANOVA with Bonferroni post-hoc test was used. To determine the ability of BC to predict perioperative outcome, uni- and multivariable logistic regression analysis was performed. The Spearman correlation coefficient was used to study the association between BC and various clinical parameters. The prognostic value of the BAR score and the new proposed

"BAR-Myosteatosis" score (including myosteatosis) for 90-day outcome was assessed using the receiver operating characteristic (ROC) curve analysis calculating the area under the receiver operating characteristic curve (AUROC). This new score was calculated by adding points for myosteatosis to the standard BAR score [8]. The Hosmer-Lemeshow chi^2 goodness-of-fit test was applied to test model suitability. All p-values<0.05 were considered statistically significant. Statistical analysis has been performed using SPSS Statistics v24 (IBM Corp., Armonk, NY, USA).

RESULTS

Recipient and donor characteristics

Out of all 357 consecutive OLTs performed within the given time frame, 225 patients met the predefined inclusion and exclusion criteria. Among 132 excluded patients were recipients of living related (n=5) or split liver allografts (n=4), and cases without sufficient preoperative CT imaging including the L3 level within the last 6 months (n=123).

The mean donor- and recipient ages were 55±16 and 54±12 years respectively. Sixty-seven per cent of the recipients were male and the most common reasons for OLT were hepatocellular carcinoma (28%) and alcoholic liver cirrhosis (20%) followed by acute liver failure (14%). The mean pre-OLT laboratory MELD score was 20±11, whereas the mean BAR and SOFT scores were 9±6 and 15±10 respectively. The mean recipient BMI was 27±5. Further patients' characteristics are shown in Table 1.

Body composition analysis

The median time between the CT-imaging used for segmentation and OLT was 5 weeks (range 0-24). The mean SMI was 57±39 cm^2/m^2 for male and 47±11 cm^2/m^2 for female patients. The mean SM-RA were 35±11 HU for male and 32±11 HU for female, while VFA were 130±92 cm^2 for male and 94±156 cm^2 for female, respectively. The main BC profiles calculated from the CT analysis and the stratification of the patient cohort based on the specific cut-off values are summarized in Table 2. Males had slightly but significantly higher SMI, SM-RA, VFA than females (p<0.05). Briefly, based on the aforementioned cut-offs, 84 patients showed reduced muscle mass and 98 had myosteatosis, indicating a higher incidence of myosteatosis than reduced SMM only. Some 103 patients have been found to have visceral obesity and 34 were sarcopenic obese (Table 2). The used body composition parameters have showed weak to moderate strong correlations with various recipient characteristics including recipient age, BMI, labMELD and Child-Pugh scores, pre-transplant Albumin levels and PLT counts (Supplementary table 1A). SMI only showed significant moderate correlation with recipient BMI (r=0.466, p<0.001). Negative associations were found between SM-RA and recipient pre-transplant labMELD and Child-Pugh scores (labMELD: r=-0.403, p<0.001; Child-Pugh: r=-0.338, p<0.001; Supplementary table 1A). Recipient pre-OLT functional status (KPS) has showed a moderate strong

association with the SM-RA values (r=0.438, p<0.001, Supplementary table 1A). Accordingly, KPS was significantly lower in patients with myosteatosis compared to the rest of the cohort (50±20 vs. 70±20, p<0.001).

Table 2. Stratification of the cohort based on body composition features

Categories		No. of patients (n=225)	%
Skeletal muscle mass (SMI)	No	141	63
	Yes	84	37
Myosteatosis (SM-RA)	No	127	56
	Yes	98	44
Visceral obesity (VFA)	No	122	54
	Yes	103	46
Sarcopenic obesity	No	191	85
	Yes	34	15

Abbreviations used: SMI: lumbar 3 skeletal muscle index, SM-RA: lumbar 3 skeletal muscle radiation attenuation, VFA: lumbar 3 visceral fat area.

Perioperative outcome and morbidity

One hundred and fourteen (n=114) patients (51%) developed major complications (CD≥3b) within 90 days after OLT (Table 3). The mean CCI score was 54±33 (Table 3). Among all BC parameters tested, only SM-RA showed statistically significant and clinically meaningful AUROC values (AUROC≈0.7) for major morbidity (CD≥3b; SM-RA Male, 0.678 AUROC; SM-RA Female, 0.700 AUROC, p<0.001, Supplementary table 1B). Significantly more patients in the myosteatosis group had major complications compared to the recipients with normal muscle density (67% vs. 38%, p<0.001) accompanied with a higher 90-day mortality rate in these patients (15% vs. 3%, p=0.001, Table 3).

Accordingly, patients with myosteatosis also had markedly higher CCI scores (68±32 vs. 44±30; p<0.001) (Table 3). The incidence of EAD was significantly higher (36% vs. 20%, p=0.006, Table 3). Myosteatotic patients received significantly more transfusion of RBC and FFP units intraoperatively (RBC: 12±10 vs. 7±6 units, p<0.001; FFP 20±12 vs. 16±7 units, p<0.001, respectively; Table 3). Similar to myosteatosis, sarcopenic obesity was also associated with an increased need of intraoperative transfusions (Table 3).

Table 3. Perioperative outcome stratified by body composition

Reduced skeletal muscle mass	**All patients**	**No**	**Yes**	***p*-value**
	n=225	**n=141**	**n=84**	
90-day [3]CD3b complications[1] n (%)	114 (51)	70 (50)	44 (52)	0.890
90-day mortality n (%)	19 (8)	12 (9)	7 (8)	0.959
Early allograft dysfunction[2] n (%)	60 (27)	38 (27)	22 (26)	0.877
ICU stay (days)	14±23	13±20	16±27	0.101
Hospital stay (days)	43±42	39±29	50±58	0.815
Intraoperative RBC transfusion (units)	9±8	9±7	10±10	0.386
Intraoperative FFP transfusion (units)	18±10	17±8	18±13	0.690
90-day CCI[3]	54±33	53±32	56±34	0.663
Cost estimation (TEuro)[4]	60±27	59±27	60±27	0.721
Myosteatosis	n=225	n=127	n=98	
90-day [3]CD3b complications n (%)	114 (51)	48 (38)	66 (67)	0.000
90-day mortality n (%)	19 (8)	4 (3)	15 (15)	0.001
Early allograft dysfunction n (%)	60 (27)	25 (20)	35 (36)	0.006
ICU stay (days)	14±23	11±21	18±25	0.000
Hospital stay (days)	43±42	33±24	56±55	0.000
Intraoperative RBC transfusion (units)	9±8	7±6	12±10	0.000
Intraoperative FFP transfusion (units)	18±10	16±7	20±12	0.000
90-day CCI	54±33	44±30	68±32	0.000
Cost estimation (TEuro)	60±27	50±22	72±29	0.000
Visceral obesity	n=225	n=122	n=103	
90-day [3]CD3b complications n (%)	114 (51)	57 (47)	57 (55)	0.271
90-day mortality n (%)	19 (8)	11 (9)	8 (8)	0.812
Early allograft dysfunction n (%)	60 (27)	30 (25)	30 (29)	0.469
ICU stay (days)	14±23	14±20	14±25	0.671
Hospital stay (days)	43±42	42±31	45±53	0.541
Intraoperative RBC transfusion (units)	9±8	9±7	10±9	0.534
Intraoperative FFP transfusion (units)	18±10	17±9	18±11	0.241
90-day CCI	54±33	54±34	55±32	0.846
Cost estimation (TEuro)	60±27	59±28	60±26	0.681
Sarcopenic obesity	n=225	n=191	n=34	
90-day [3]CD3b complications n (%)	114 (51)	94 (49)	20 (59)	0.227
90-day mortality n (%)	19 (8)	16 (8)	3 (9)	0.743
Early allograft dysfunction n (%)	60 (27)	49 (26)	11 (32)	0.377
ICU stay (days)	14±23	14±21	16±30	0.952
Hospital stay (days)	43±42	39±29	64±81	0.344
Intraoperative RBC transfusion (units)	9±8	9±7	13±12	0.015
Intraoperative FFP transfusion (units)	18±10	17±8	22±15	0.031
90-day CCI	54±33	53±33	62±33	0.163
Cost estimation (TEuro)	60±27	59±27	65±27	0.142

[1]Refers to Clavien et al.[30]; [2]Refers to Olthoff et al.[28]; [3]Refers to Slankamenac et al.[29]; [4]Refers to Staiger et al.[31]

Abbreviations used: CD: Clavien-Dindo classification, ICU: intensive care unit, RBC: red blood cell units, FFP: fresh frozen plasma units, CCI: Comprehensive Complication Index, TEuro: thousand Euros

The mean number of days, that patients spent on the ICU- and in hospital were 14±23 days and 43±42 days respectively (Table 3). Patients with myosteatosis stayed significantly longer on ICU (18±25 vs. 11±21 days, p<0.001) and had a significantly longer total hospital stay (56±55 vs. 33±24 days, p<0.001) compared to patients over the defined SM-RA cut-offs (Table 3). Accordingly, the mean procedural costs were considerably higher in cases with preoperative myosteatosis (72±29 vs. 50±22 TEuro, p<0.001, respectively; Table 3). With regards to hospital stay, a substantial but statistically non-significant difference was noted between sarcopenic obese patients and the rest of the cohort (64±81 vs. 39±29 days, p=0.344; Table 3). Interestingly, neither the presence of reduced skeletal muscle mass alone nor visceral obesity affected significantly any of the above-described outcome parameters (Table 3).

In line with the findings above, a significant association was observed between the SM-RA values and the days spent on ICU and in hospital, as well as with CCI score and estimated costs (ICU stay: r=-0.392, p<0.001; Hospital stay: r=-0.333, p<0.001; CCI score: r=-0.373, p<0.001; Costs: r=-0.400, p<0.001; Table 4). Neither SMI, nor VFA showed a significant correlation with any of these parameters (Table 4).

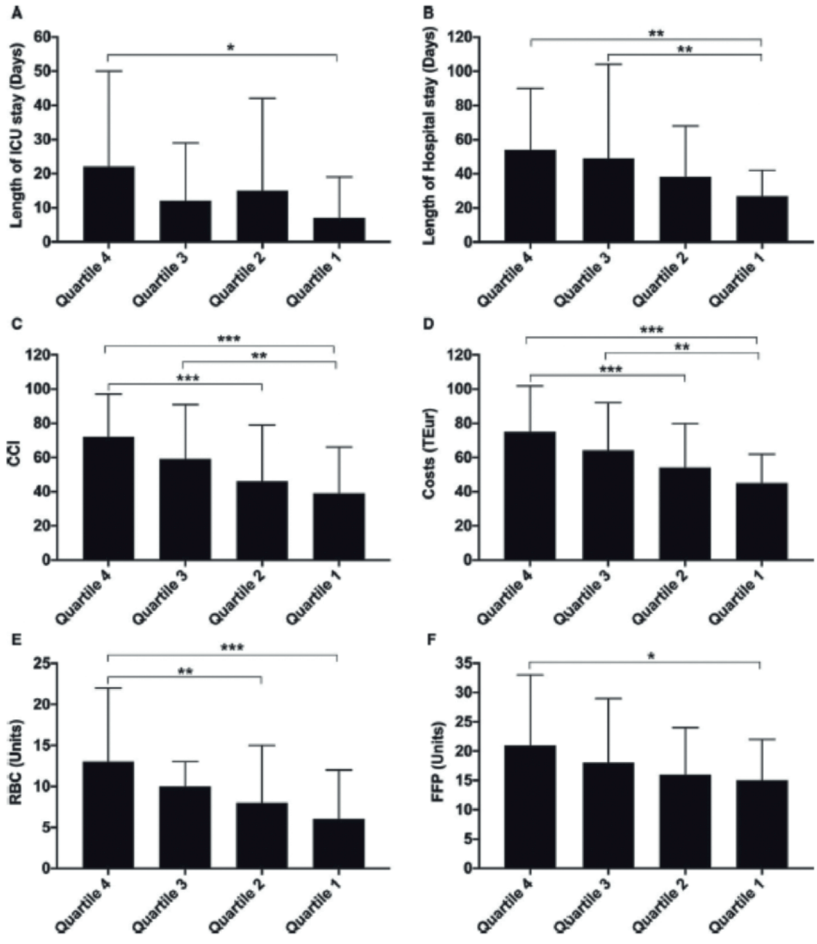
Table 4. Correlation of outcome with body composition parameters

Body composition	ICU stay		Hospital stay		CCI		Costs	
	r	p	r	p	r	p	r	p
SMI (cm2/m2)	0.037	0.584	-0.011	0.871	-0.011	0.868	-0.013	0.847
SM-RA (HU)	-0.392	0.000	-0.333	0.000	-0.373	0.000	-0.400	0.000
VFA (cm2)	-0.025	0.708	-0.074	0.267	-0.002	0.982	0.024	0.721

Abbreviations used: SMI: lumbar 3 skeletal muscle index, SM-RA: lumbar 3 skeletal muscle radiation attenuation, VFA: lumbar 3 visceral fat area.

To further analyse the effects of myosteatosis on perioperative outcomes, patients were divided into quartiles based in the SM-RA values. This lead to a gradual reduction of ICU- and hospital stay, CCI scores, mean procedural costs, and the need of intraoperative transfusions between the subgroups of patients belonging to the various quartiles. There was a significant difference between the group of patients in the first- and last quartiles in each examined parameter (Quartile 1 vs. 4; ICU stay, 7±12 vs. 22±28 days, p=0.004; Hospital stay, 27±15 vs. 54±36 days, p=0.001; 90-days CCI score, 39±27 vs. 72±25, p<0.001; Estimated procedural costs, 45±17 vs. 75±27 TEur, p<0.001; Intraoperative transfusion of RBC units, 6±6 vs. 13±9 units, p<0.001; Intraoperative transfusion of FFP units, 15±7 vs. 21±12 units, p=0.026; Figure 2).

Figure 2. Perioperative outcome and procedural costs stratified by the quartiles of skeletal muscle radiation attenuation



Length of intensive care unit stay (A), length of hospital stay (B), Comprehensive Complication Index (C), procedural costs (D), intraoperative transfusion of red blood cell units (E), and intraoperative transfusion of fresh frozen plasma units (F) grouped according to the quartiles of skeletal muscle radiation attenuation values (SM-RA). (mean±standard deviation, *p<0.05, **p<0.01, ***p<0.001, one-ways ANOVA and Bonferroni post-hoc test, n=54, 58, 57, 56, respectively)

Predictors of ninety-day morbidity

Univariable logistic regression analysis showed that pre-transplant Child-Pugh Score, labMELD, pre-transplant ICU stay, KPS, and myosteatosis were significantly associated with major postoperative morbidity (Clavien-Dindo ≥3b) (Table 5).

In the multivariable analysis, myosteatosis (OR 2.772, 95%CI 1.516-5.066, p=0.001) remained significant and have been identified as an independent predictor of major morbidity following OLT (Table 5).

Table 5. Uni- and multivariable logistic regression analysis for 90-days major morbidity (Clavien-Dindo ³3b)

	Major complications (CD ³3b)[1] n=114	No- / minor complications (CD1-3a)[1] n=111	Univariable analysis		Multivariable analysis	
			Odds-ratio (95% Confidence Interval)	*p value	Odds-ratio (95% Confidence Interval)	p value
Donor age ³60 years	49 (43)	43 (39)	1.175 (0.688-2.006)	0.554		
Donor BMI ³25	91 (80)	85 (77)	1.168 (0.610-2.236)	0.640		
Donor Sex Male	63 (55)	54 (49)	1.331 (0.785-2.258)	0.288		
Pre-transplant Child-Pugh Score ³7	80 (70)	60 (54)	1.889 (1.091-3.269)	0.023	0.942 (0.464-1.912)	0.868
ECD[2] Yes	80 (70)	74 (67)	1.099 (0.625-1.932)	0.743		
Recipient age ³60 years	44 (39)	42 (38)	1.017 (0.593-1.746)	0.951		
Recipient BMI ³25	84 (74)	68 (61)	1.746 (0.985-3.098)	0.057		
Recipient Sex Male	77 (68)	73 (66)	1.056 (0.604-1.845)	0.849		
Etiology of liver disease				0.314		
ALF	13 (11)	18 (16)	0.628 (0.247-1.594)			
HCC	28 (25)	35 (31)	0.671 (0.307-.1.466)			
Alcoholic cirrhosis	30 (26)	15 (14)	1.863 (0.779-4.459)			
Viral	6 (5)	9 (8)	0.580 (0.176-1.914)			
PSC/PBC	10 (9)	11 (10)	0.791 (0.278-2.248)			
Graft failure	2 (2)	2 (2)	0.870 (0.112-6.751)			
Other	25 (22)	21 (19)	1			
Pre-transplant labMELD ³25	53 (46)	21 (19)	3.447 (1.886-6.301)	0.000	2.340 (0.803-6.820)	0.119
Recipient pre-transplant ICU Yes	40 (35)	16 (14)	3.434 (1.761-6.694)	0.000	1.867 (0.699-4.985)	0.213
Recipient pre-transplant abdominal surgery Yes	43 (38)	39 (35)	1.105 (0.639-1.913)	0.721		
Recipient pre-transplant encephalopathy Yes	50 (44)	40 (36)	1.425 (0.830-2.444)	0.199		
Karnofsky Performance Score <60	58 (51)	30 (27)	2.975 (1.687-5.247)	0.000	0.794 (0.242-2.609)	0.704
Cold ischemic time ³480 (min)	65 (57)	58 (52)	1.245 (0.728-2.129)	0.423		
Warm ischemic time ³45 min	53 (46)	60 (54)	0.728 (0.427-1.243)	0.245		
Reduced skeletal muscle mass (SMI) Yes	43 (38)	41 (37)	1.060 (0.615-1.826)	0.835		
Myosteatosis (MA) Yes	66 (58)	32 (29)	3.407 (1.949-5.958)	0.000	2.772 (1.516-5.066)	0.001
Visceral obesity (VFA) Yes	56 (49)	47 (42)	1.346 (0.792-2.285)	0.272		
Sarcopenic obesity Yes	20 (18)	14 (13)	1.588 (0.747-3.376)	0.229		

Values were given as mean±standard deviation or numbers and (per cent). Results of the logistic regression were given as odds-ratios with 95% confidence interval.
*Factors showing significant results in the univariable analysis were included into the multivariable logistic regression model. Only significant results are shown. To avoid a multicollinearity effect, certain variables were not included into the logistic regression analysis.

[1]Refers to Clavien et al.[30]; [2]Refers to German Medical Chamber Guidelines.[27]

Abbreviations used: BMI: body mass index, ECD: extended criteria donor allografts, ALF: acute liver failure, HCC: hepatocellular carcinoma, PSC: primary sclerosing cholangitis, PBC: primary biliary cirrhosis, MELD: model for end-stage liver disease, CCI: comprehensive complication index, ICU: intensive care unit, SMI: lumbar 3 skeletal muscle index, SM-RA: lumbar 3 skeletal muscle radiation attenuation, VFA: lumbar 3 visceral fat area.

Combining myosteatosis with a validated prediction model: "the BAR-Myosteatosis" score

The prognostic value of the original BAR score and the newly proposed BAR-Myosteatosis score have been compared based on the AUROC values calculated for 90-days major morbidity and mortality. Accordingly, the AUROC increased gradually from 0.677 and 0.821 (original BAR score) to 0.710 and 0.853 by adding points to the BAR score for patients with preoperative myosteatosis, confirming a potential added value of myosteatosis as a predictor of early outcomes (Table 6).

Table 6. The "BAR-Myosteatosis" score

Variable	AUC	SE	95% CI	p-value	chi²*	p-value#
90-day major morbidity (³CD3b)						
BAR	0.677	0.036	0.606-0.748	0.000	5.011	0.659
BAR-Myosteatosis (2 pts)	0.695	0.035	0.625-0.764	0.000	6.276	0.616
BAR-Myosteatosis (4 pts)	0.704	0.035	0.635-0.772	0.000	9.812	0.279
BAR-Myosteatosis (6 pts)	0.706	0.035	0.638-0.775	0.000	9.908	0.194
BAR-Myosteatosis (8 pts)	0.710	0.035	0.642-0.778	0.000	7.245	0.510
90-day mortality						
BAR	0.821	0.060	0.702-0.939	0.000	3.363	0.850
BAR-Myosteatosis (2 pts)	0.838	0.052	0.736-0.941	0.000	6.645	0.575
BAR-Myosteatosis (4 pts)	0.848	0.046	0.757-0.939	0.000	5.300	0.725
BAR-Myosteatosis (6 pts)	0.855	0.041	0.774-0.935	0.000	3.231	0.863
BAR-Myosteatosis (8 pts)	0.853	0.039	0.777-0.929	0.000	6.714	0.568

*Hosmer-Lemeshow chi²; # in case of a p-value of <0.05 the test rejects the null hypothesis of an adequate fit.

Abbreviations used: BAR: Balance of Risk, CD: Clavien-Dindo classification, AUC: area under the curve, SE: standard error, CI: confidence interval

DISCUSSION

The present study indicates that BC alterations are highly prevalent in patients with terminal liver disease. While more than 40% of our recipients presented with myosteatosis before OLT, we show here for the first time the superior prognostic value of myosteatosis (muscle quality) over reduced skeletal muscle mass only (morphological manifestation of sarcopenia) in predicting perioperative outcomes in deceased donor OLT. Myosteatosis was significantly associated with poor perioperative outcome including transfusion of blood and blood products, morbidity, mortality, EAD, ICU- and hospital stay. The estimated procedural costs over the first 90-days when transplanting patients with myosteatosis were significantly higher and myosteatosis was identified as an independent predictor of major surgical complications.

Body composition can be assessed using different methods including bioelectrical impedance analysis (BIA), air displacement plethysmography, and dual-energy X-ray

absorptiometry [2, 15]. The sensitivity of these techniques to differentiate between muscle quantity and quality is, however, limited and often biased by the significant amount of fluid overload in patients with terminal liver disease [2]. In these patients, the clinical estimation of BC and the assessment of their physical strength using conventional parameters (body weight, waist circumference, BMI, ability to walk or physical activity, handgrip) is usually difficult and often compromised by the presence of ascites and/or encephalopathy. Therefore, CT-imaging is currently the gold standard for the quantification of muscle mass and muscle quality in patients with terminal liver disease [2, 16, 20].

While some studies have suggested a potential role of myosteatosis in OLT, all of these reports were focusing predominantly on sarcopenia and/or living donor liver transplantation [2, 15, 22, 35, 36]. The specific effect of myosteatosis on the perioperative outcomes of deceased donor OLT remains to be determined. In our cohort, patients with myosteatosis had significantly more major complications over the first 3 months and low muscle density was additionally associated with increased rates of EAD, higher CCI scores and an increased need for intraoperative blood transfusions. In this regard it should be noted, that a significantly prolonged ICU- and hospital stay may also reflect an increased surgical morbidity and an overall delayed functional recovery in these patients. The estimated mean procedural costs were more than twenty-thousand Euros higher in patients with myosteatosis compared to the rest of our cohort. In myosteatosis, an increasing intramuscular fat accumulation (Figure 1) is associated with a pro-inflammatory microenvironment that potentially impairs immune function via proinflammatory cytokines and adipocytokines [35, 36]. These humoral factors and muscle-to-liver cross-talk, together with the limited functional reserves in myosteatotic patients (e.g. reduced KPS score, greater need of intraoperative transfusions) may contribute to an increased oxidative stress and graft injury with a higher incidence of EAD during the perioperative phase [19, 37, 38].

The association of myosteatosis with the above-mentioned parameters of perioperative outcome is further supported by the correlation and quartile analyses, demonstrating that gradually decreasing muscle density leads to more perioperative complications, longer in-hospital stay and higher estimated costs. Of note, reduced SMM, visceral obesity and sarcopenic obesity were not associated with increased morbidity in our cohort.

The most interesting observation of the present study is the superiority of myosteatosis (muscle quality) over skeletal muscle mass alone (muscle quantity as morphological aspect of sarcopenia) in predicting perioperative outcomes following OLT. Although, earlier studies investigated changes in skeletal muscle mass to define sarcopenia, recent evidence suggests that fat accumulation within the muscle might be responsible for functional impairment and negative pathophysiological responses even in patients with quantitatively normal or nearly normal muscle mass [15]. In a recent report by Hamaguchi et al., the authors demonstrated the association of psoas muscle mass index

and intramuscular adipose tissue content index (IMAC) with post-transplant survival in a single-centre Japanese cohort of 200 recipients of living donor liver transplantation [35]. Furthermore, Bhanji et al. have reported their observation in 675 cirrhotic patients and reported a significant association of myosteatosis with hepatic encephalopathy and mortality on the OLT waiting list [21]. Although, myosteatosis has been linked to inferior survival in cirrhotic patients, the prognostic role of myosteatosis in post-OLT outcomes in deceased donor OLT remains to be determined [2, 21, 22, 39]. In our study, myosteatosis was identified as the only and most important predictor of post-OLT morbidity. Although, other relevant factors showed significant results in our univariable analysis (e.g. labMELD, Child-Pugh Score, pre-OLT ICU stay), they have lost their significant effect as independent predictors in the multivariable analysis. A possible explanation is the inherent complexity and heterogeneity of OLT cohorts in general, with a high number of factors potentially affecting clinical outcomes (especially in terms of perioperative outcome). Given that high-MELD patients (>30) usually present with worse clinical outcome and higher complications rates, a relatively low MELD-score within our cohort (mean labMELD score 20±11) may be the reason why MELD has lost its significance in multivariable analysis.

Myosteatosis is believed to occur when lipids intake exceeds the disposal capacity of the adipose tissue and muscle, functioning as a form of ectopic fat storage in overweight subjects [19, 40]. While, myosteatosis is also seen in normal weight or underweight patients [41], it is reasonable to assume that the skeletal muscle does not only act as a plain ectopic storage of energy surplus and as such mechanisms other than excessive fat intake may play an important role in the development of pathological muscle fat deposition [19]. In our study, myosteatosis was moderately but significantly associated with the severity of the underlying liver disease (labMELD, Child-Pugh Score, Albumin, platelet count), highlighting the importance of a complex and poorly understood liver-to-muscle cross-talk over a simple nutritional theory [19].

Several clinical risk-assessment tools have been developed for assessing outcome following OLT [13]. Out of these, the BAR score, a promising tool developed by Dutkowski et al., includes easily available donor and recipient risk factors with the aim to clinically assess and predict the risk of poor post-OLT outcomes [8, 14]. Based on an excellent stratification of patients at risk using myosteatosis, we propose a new "BAR-Myosteatosis" score adding myosteatosis as an adjunct parameter to the original BAR score. Interestingly, this novel score demonstrated superior AUROC values for perioperative morbidity and mortality compared to the original BAR score.

The findings of this study should be interpreted in the light of potential limitations. First, due to the inherit retrospective, single-center and uncontrolled nature of our analysis, no or very limited preoperative functional assessment of patient fitness (except Karnofsky PS) and nutritional status was possible. Second, we could not compare the performance of CT-based BC with other methods and our evaluation did not include the routine assessment of muscle strength, such as handgrip, gait speed or 6-minute walk

tests [22]. While some centers may prefer the use of magnetic resonance imaging (MRI) over CT for cross-sectional imaging in OLT candidates, future studies should explore the possibilities of MRI-based assessment of BC and myosteatosis. Even though van Dijk et al. recently confirmed that myosteatosis may be adequately assessed using either MRI or CT scans in a group of patients with periampullary cancer [42], the prognostic value of MRI-based BC-assessment for patients with end-stage liver disease remains to be determined. Based on current evidence, MRI-based BC-assessment is therefore not yet implemented in current EASL and AASLD guidelines [15, 16, 18].

Notwithstanding the aforementioned limitations, we have identified myosteatosis as an independent marker of prognosis in patients undergoing OLT. In addition, the superior prognostic value of muscle quality (myosteatosis as assessed by the SM-RA) over quantity (SMM as assessed by the SMI) in predicting perioperative outcomes does not only support the role of nutritional screening and therapeutic interventions in patients undergoing OLT, but also emphasizes the clinical relevance of myosteatosis in OLT risk-assessment. Further research on the underlying molecular mechanisms of myosteatosis in OLT and external validation of our findings in prospective biomarker embedded clinical trials are warranted. Future trials should also incorporate therapeutic interventions such as the monitoring of dietary intake, protein or branched-chain amino acid supplementation, pre-habilitation, physical exercise and/or pharmaco-therapy.

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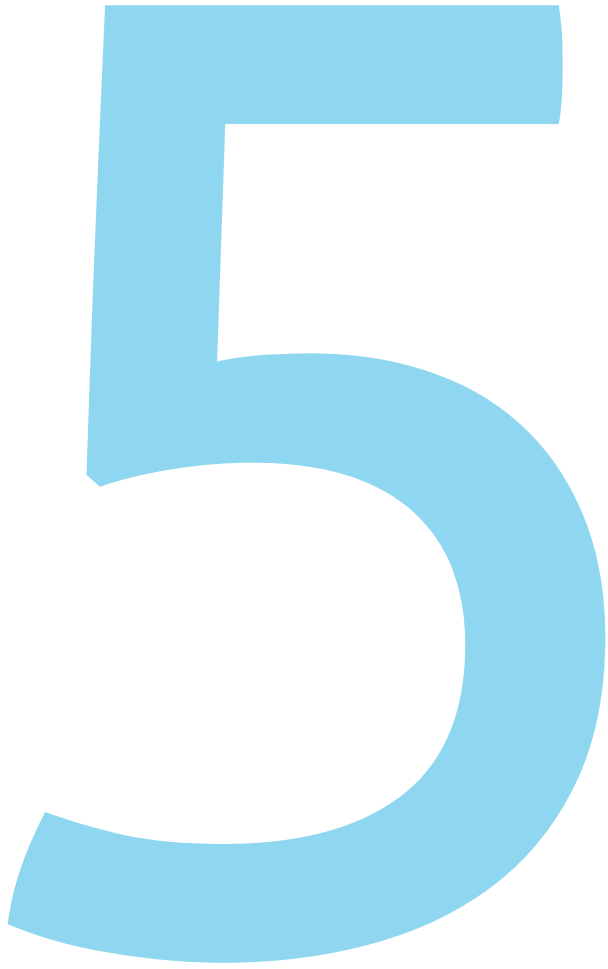
CHAPTER



Low Thoracic Muscle Radiation Attenuation is associated with Postoperative Pneumonia Following Partial Hepatectomy for Colorectal Metastasis

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ABSTRACT

Background: Low skeletal muscle radiation attenuation (SM-RA) is indicative of myosteatosis and diminished muscle function. It is predictive of poor outcome following oncological surgery in several cancer types. Postoperative pneumonia is a known risk factor for increased postoperative mortality. We hypothesized that low SM-RA of the respiratory muscles at the 4th thoracic-vertebra (T4) is associated with postoperative pneumonia following liver surgery.

Methods: Postoperative pneumonia was identified using prospective infection control data. Computed tomography body composition analysis was performed at the L3- and T4 level to determine SM-RA. Body composition variables were corrected for confounders and related to postoperative pneumonia and admission time by multivariable logistic regression.

Results: Body composition analysis of 180 patients was performed. Twenty-one patients developed postoperative pneumonia (11.6%). Multivariable analysis showed that low T4 SM-RA as well as low L3 SM-RA were significantly associated with postoperative pneumonia (OR 3.65, 95% CI 1.41-9.49, $p<0.01$) and (OR 3.22, 95% CI 1.20-8.61, $p=0.02$, respectively).

Conclusion: Low SM-RA at either the L3- or T4-level is associated with a higher risk of postoperative pneumonia following CLRM resection. T4 SM-RA reflecting thoracic myosteatosis may be a valuable body composition parameter in the context of preoperative risk stratification when no abdominal scan is available.

INTRODUCTION

The impact of sarcopenia and muscle wasting on the efficacy of treatment and outcome following oncological surgery has been widely established (1-4). Sarcopenia has been shown to be associated with reduced pulmonary function in healthy adults, as well as with increased pulmonary complications following oncological esophageal resection (5-9). In addition, skeletal muscle radiation attenuation (SM-RA) has emerged as a radiological marker indicative of myosteatosis, which has been shown to be predictive of poor outcome following oncological surgery (2, 10, 11). Myosteatosis is characterized by increased inter- and intra-myocellular fat stores, which can be influenced by dietary pattern and activity changes. This is related to reduced physical fitness and reduced muscle function (12-16).

Postoperative pneumonia has been shown to increase postoperative morbidity, prolong hospital admission, and increase in-hospital mortality following a range of surgical interventions, most notably after major abdominal and upper gastro-intestinal surgery (17-21). More specifically, patients undergoing partial hepatectomy frequently develop reactive pleural effusion, which increases the risk of post-operative pneumonia to incidences above 10% (17, 19-24). Besides the medical implications, pulmonary complications constitute a significant burden to healthcare systems by increasing healthcare costs (19, 22).

Recent developments in perioperative patient conditioning have suggested that prehabilitation training can increase preoperative aerobic condition, thereby reducing postoperative pneumonia following major abdominal surgery (25). To that end, identifying patients at risk for postoperative pneumonia is of paramount importance.

Bearing in mind that SM-RA is indicative of muscle function, we hypothesized that low SM-RA is indicative of increased risk of postoperative pneumonia, and that SM-RA predicts postoperative pneumonia better than muscle mass. Furthermore, we hypothesized that SM-RA assessed at the 4th thoracic vertebra (T4) predicts postoperative pneumonia better than SM-RA measurements at the 3rd lumbar vertebra (L3), when assuming that it reflects *thoracic* muscle myosteatosis.

Thus, the aims of this study were 1) to assess whether reduced SM-RA is associated with increased postoperative pneumonia, and 2) to investigate whether T4 SM-RA has a stronger association with postoperative pneumonia than L3 SM-RA.

MATERIALS AND METHODS

Study design

Prospective infection control data of a cohort of patients aged 18 years and older who underwent elective CRLM resection between January 2008 and December 2013 at Maastricht University Medical Center were collected. Exclusion criteria included American Society of Anesthesiology (ASA)-classification V, severe liver cirrhosis (Child grade C), end-

stage renal disease requiring dialysis, severe heart disease (New York Heart Association class IV) and chronic obstructive pulmonary disease (COPD) requiring (home)oxygen therapy. Furthermore, patients were excluded from analysis if their CT scans did not show the thoracic or abdominal wall or displayed large radiation artifacts, if they had a time interval greater than three months between the time of the scan and surgery, and if the thoracic and abdominal scans were not performed at the same time.

Besides body composition data, sex, age, ASA-classification (<3 and ≥3), smoking, planned type of procedure (laparoscopic or open), comorbidities, and body mass index (BMI), Portal Vein Embolization (PVE), neoadjuvant chemotherapy, tumor stage (1-2 versus 3-4), duration of surgery, minor versus major liver resection (<3 and ≥3 liver segments), and left- versus right-sided liver resections were considered potential confounders (26, 27). Right-sided resections were hypothesized to have an increased risk of pleural effusion and thus an increased risk of pneumonia (24).

The primary endpoint was the occurrence of postoperative pneumonia as defined below. The secondary outcomes were length of hospital stay, in hospital mortality, and 30-day mortality. In addition, we evaluated the association with major abdominal complications rather that selectively evaluating postoperative pneumonia, to explore whether pneumonia was a surrogate for major abdominal complications, and whether reduced SM-RA was associated with major complications. Data was reported in accordance with STROBE guidelines for reporting of observational COHORT studies (28).

Defining postoperative pneumonia

Pulmonary infection data were prospectively acquired by a study nurse in collaboration with clinicians and infection specialists in the context of compliance to national quality control guidelines. The criteria for respiratory infection were set as a fever greater than 38 degrees Celsius, chest pain, dyspnea and cough including expectoration developing within 30 days of surgery in addition to an infiltration visible on the chest x-ray, regardless of the presence or absence of bacteria in the sputum (29). Because these data were ascertained in the context of a quality control evaluation, no blinding was performed. Clinical evaluation was performed by the treating surgeon and the evaluation of the chest x-ray was performed by the on-duty radiologist.

Defining major abdominal complications

We used the liver surgery-specific composite endpoint (LSSCEP) composed of postoperative liver failure, bile leakage, intra-abdominal hemorrhage, intra-abdominal abscess, and mortality to assess liver surgery-specific complications (30). Liver failure was defined according to the peak bilirubin criterion of Mullen et al (31). Patients were defined as having suffered a major abdominal complication if at least one LSSCEP event had occurred.

CT body composition analysis

Muscle mass was assessed by analyses of electronically stored CT images; these CT scans were part of routine clinical work. Body composition analysis of abdominal and thoracic CT scans was performed. CT scans were selected and analyzed blindly by a single investigator using Slice-O-matic software, version 5.0 (Tomovision, Montreal, QC, Canada).

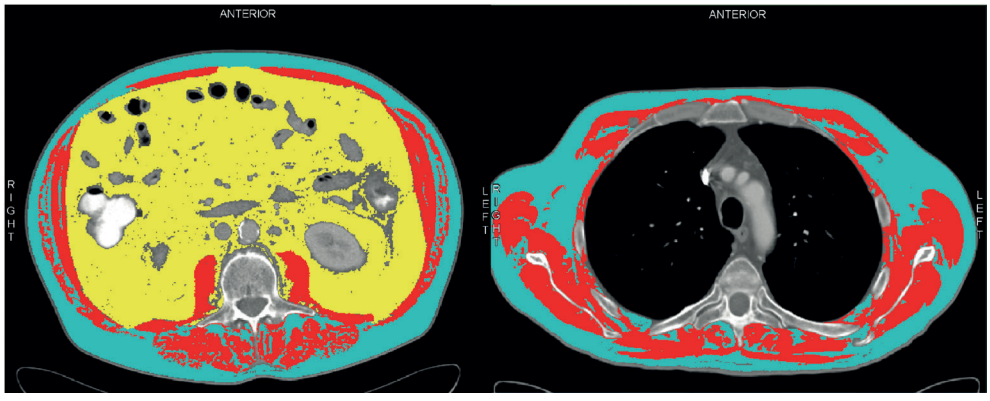
Abdominal body composition measurements

L3 was used as a standard landmark to measure tissue cross-sectional area in cm^2 as previously reported (2). In short, skeletal muscle (SM), visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT) were identified and quantified on CT images using predefined Hounsfield unit (HU) ranges (SM: -29 to 150 HU, VAT: -150 to -50 HU, and SAT: -190 to -30 HU) (32). SMA, VAT, and SAT were corrected for stature to calculate the L3-muscle index, L3-VAT index, and L3-SAT index in cm^2/m^2, providing good estimates of total body SM, VAT, and SAT mass (32). Cut offs were established based on tertiles (see statistical analysis) (Table 2).

Thoracic body composition measurements

T4 was used as a standard landmark to measure tissue cross-sectional area in cm^2. SMA and SAT were identified and quantified on CT images using the same predefined Hounsfield unit (HU) ranges as in the abdominal scans (Figure 1). The total areas of SMA and SAT were corrected for stature to calculate the T4-muscle index (T4-muscle-index and T4-SAT-index).

Figure 1. Body composition segmentation



CT image of one patient suffering from myosteatosis at the L3 (left) and T4 (right) level, with muscle groups and fat segmentation: red (muscle), blue (subcutaneous fat) and yellow (visceral fat)

Muscle radiation attenuation

The radiation attenuation for skeletal muscle (SM-RA) at L3 and T4 level was assessed by calculating the average HU value of the total muscle area within the specified range of -29 to 150 HU (excluding intramuscular adipose tissue).

Statistical Analyses

Cut-offs

Previously published cut-off values for L3-muscle-index were established in cohorts of patients with different demographics and ethnicities, and may not be comparable with the present Dutch cohort of patients with CRLM (26, 33-35). Therefore, we decided to determine our own cut-offs for L3-muscle-index, as performed by other studies with similar population sizes (2, 36). Due to the fact that no consensus cut-offs exist for thoracic muscle mass, abdominal SM-RA, and thoracic SM-RA, we also stratified these parameters using cut-offs based on the current cohort.

We considered our cohort too small for cut-point analysis by optimal stratification, and therefore determined cut-off values based on tertiles stratified by sex (37). Determining the cut-off at a tertile enables comparison between groups with a relatively low/high value to be compared with the rest of the group while not forcing subjects with a value around the cut-off value in a low or high category. Cut-off values were set at the lowest tertile for all body composition variables (Table 2).

Univariable analysis

Univariable logistic regression analyses were used to determine the association between each individual variable and the occurrence of postoperative pneumonia and 30-day mortality. For patient characteristics, Student's t-test was used for continuous variables, with or without equal variances assumed based on Levene's test. For categorical variables, the Chi-square test was used; Fisher's exact test was used when necessary. The correlation between two continuous variables was examined with a Pearson's correlation coefficient. A p-value of <0.05 was considered significant. For the outcome of length of hospital stay, the log rank test and the cox regression model were used to determine the association between each variable and length of hospital stay. Patients that died during their hospital stay were censored.

Multivariable analysis

Variables with a p-value <0.10 in univariable analyses were included in multivariable analysis. Multivariable logistic regression analysis was used to assess the association between our study variables and the occurrence of post-operative pneumonia. Due to the limited number of events, there was limited power to perform multivariable analysis. Therefore, we opted to use stepwise backward elimination regression, with elimination set at a p-value >0.1. Multivariable cox regression analysis was used to assess the association

between the study variables and length of hospital stay in which patients that died during hospital admission were censored. All statistical analyses were performed using IBM SPSS version 24.0.

RESULTS

Patient Cohort

A total of 250 patients were included in the cohort. Body composition analysis of 180 patients could be performed. Fourteen patients were excluded on the basis of poor quality of CT scans, 21 were excluded due to scans not showing the thoracic or abdominal wall, and 20 patients were excluded due to an interval greater than three months between the time of the scan and surgery. Fifteen patients were excluded due to the abdominal and thoracic CT scans not being performed at the same time. Twenty-one patients suffered from postoperative pneumonia. In total 36 Patients suffered at least one LSSCEP event constituting a positive LSSCEP. (Appendix 3). The median hospital stay was 8 days (SD 14.8). Five patients (2,7%) died during admission, or within 30 days of surgery. Sixty-three percent of patients were male, with a mean age of 64.3 and a mean BMI of 26.3 kg/m^2. Eleven percent of resections were performed laparoscopically. The treatment and disease characteristics of the patients are listed in Table 1. No significant association between treatment or disease characteristics and the occurrence of pneumonia was observed. SM-RA was comparable between patients receiving and not receiving preoperative chemotherapy at T4 level (mean 39.4 HU (SD 6.4 HU) and (mean 38.9 HU (6.3 HU), and L3 level level (mean 34.1 HU (SD 8.3 HU) and (mean 34.2 HU(SD 8.9 HU). Mean and sex-specific cut-off values for all CT-derived body composition parameters are shown in Table 2. There was no missing data, all patients were included in the multivariable models.

Table 1. Disease characteristics

Treatment and Disease Characteristics				
	Pneumonia		P Value	Percent of total
	no	yes		
Duration of surgery (minutes) (Mean 215) *[1]	(OR 1.00, 95% CI0.99-1.01)		0.12	
Type of Resection*[1]			0.16	
minor	97	9		53.3%
major	62	12		46.7%
T-Stage			0.30	
1-2	21	3		13.3%
3-4	137	19		86.7%
Neoadjuvant chemotherapy			0.32	
no	51	4		30.5%
yes	108	17		69.5%
Right sided resection *[3]			0.55	
other	138	18		86.7%
right sides resection	21	3		13.3%
PVE (portal vein embolization) *[4]			0.60	
no	155	21		97.8%
yes	4	0		2.2%

Table showing treatment and disease characteristics and association with the primary outcome variable and percent of total. 2) Type of resection defined as minor when 2<=segments resected, major when ≥ segments resected. 3) Right sided resection includes right sides hemi-hepatectomy and extended right resection. 4) Portal vein embolization is performed to promote liver regeneration prior to surgery, ensure adequate functional liver remnant after resection. P-value calculated through Chi-square or fisher exact testing, 1) univariable binary logistic regression

Table 2. Calculated sex-specific cut-offs for body composition variables used in this study

	Units	Female cut off (mean - SD)	Male cut off (mean - SD)	Mean Cohort
L3-muscle-index	cm^2/m^2	36.8 (40.3 – 5.9)	46.6 (50.1 – 7.9)	46.5
L3-SM-RA	HU	29.4 (32.9 - 9.0)	31.0 (34.8 - 8.2)	34.1
L3-VAT-index	cm^2/m^2	23.9 (39.7 – 23.9)	46.7 (60.9 – 28.7)	53.0
L3-SAT-index	cm^2/m^2	69.9 (84.5 – 34.7)	43.9 (55.2 – 24.9)	66.1
T4-muscle-index	cm^2/m^2	51.9 (58.4 – 13.0)	65.2 (71.0 – 14.3)	66.3
T4-muscle-attenuation	HU	36.2 (38.6 – 6.9)	[1](39.7 – 6.0)	39.2
T4-SAT-index	cm^2/m^2	61.4 (76.4 – 32.2)	42.1 (53.0 - 21.0)	66.1

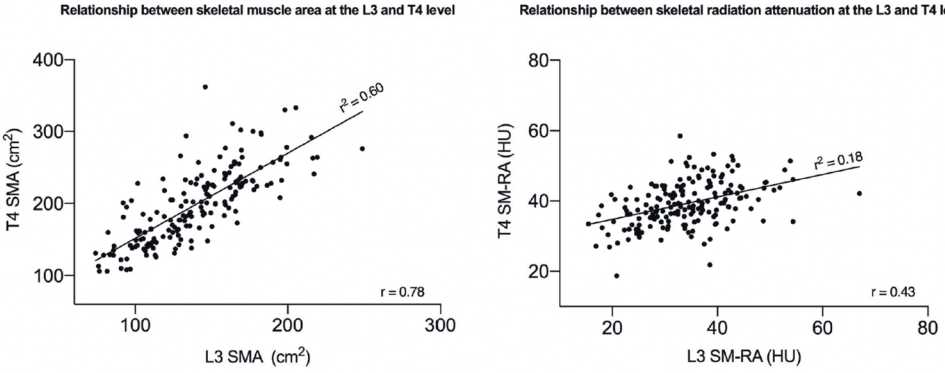
Sex-specific cut-offs and mean value based on tertiles of our cohort for each body composition variable

Thoracic and abdominal body composition imaging methods

L3 and T4 measurements of both SMA and SM-RA showed significant correlations, with T4 and L3 SMA showing a stronger correlation ($r = 0.78$, $p < 0.001$, $r^2 = 0.60$) than T4 and

L3 SM-RA (r = 0.43, p < 0.001,r^2 = 0.18) (Figure 2). Intra-observer reliability was ascertained by performing blinded re-segmentation of a subset of L3 and T4 scans (N=25). Intra-observer reliability was similar in both groups with correlation coefficient of 0.98 (95% CI 0.95 – 0.99) for T4 SM-RA and 0.99 (95% CI 0.98 – 0.99) for L3 SM-RA.

Figure 2. Relationship between skeletal muscle area (SMA) and skeletal muscle radiation attenuation (SM-RA) at the L3 and T4 level



Scatter plots for the correlation between L3- and T4 SMA (r = 0.78, p<0.01) and L3 and T4 SM-RA (r = 0.43, p<0.01). Correlation measured with Pearson correlation testing.

Univariable analysis

Univariable analysis (Appendix 1) indicated that low T4 SM-RA was associated with a high risk of postoperative pneumonia (OR 2.74, 95% CI 1.22-6.12, p=0.01). Low L3 SM-RA (OR 2.20, 95% CI 0.99-4.89, p=0.05), increased BMI (OR 0.89, 95% CI 0.78-1.02, p=0.09), and increased age (OR 1.04, 95% CI 0.99-1.09, p=0.09) were not significant, however were included in multivariable analysis.

Low L3 SM-RA (HR 1.45, 95% CI 1.05-2.01, p=0.03), low T4 SM-RA (HR 1.40, 95%CI 1.01-1.94, p=0.04), increased age (HR 0.98,95%CI 0.97-0.99, p=0.03), as well as open procedure (HR 3.16,95%CI 1.92-5.21, p<0.01) were associated with hospital stay in univariable cox-regression analysis (Appendix 2).

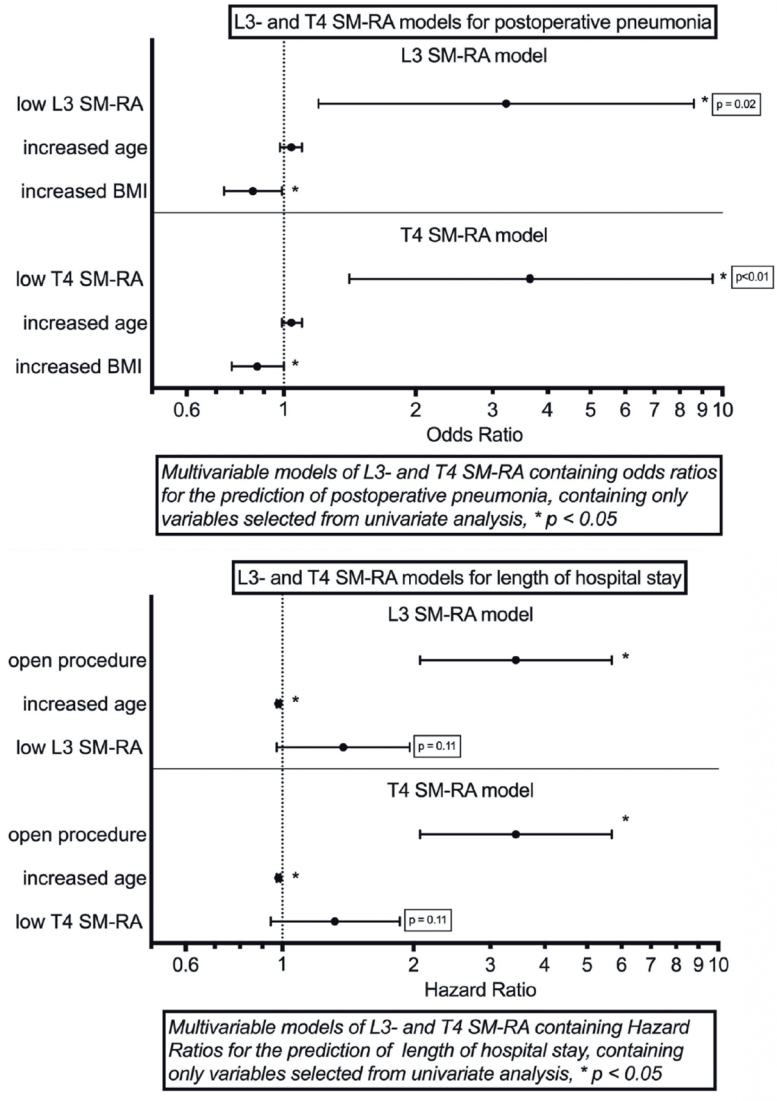
Postoperative pneumonia and a positive LSSCEP were significantly associated (p<0.01). 62% of patients with postoperative pneumonia also had positive LSSCEP (n=13). However, no significant association was found between any of our study variables and a positive LSSCEP.

Multivariable analysis

Due to co-linearity between L3 and T4 SMA and SM-RA, we created two multivariable models for the occurrence of postoperative pneumonia and increased length of hospital stay containing 1) L3 SM-RA and 2) T4 SM-RA. In both L3 and T4 multivariable models, low SM-RA remained associated with increased risk of postoperative pneumonia, when

corrected for BMI and age (Figure 3 & Appendix 1). T4 SM-RA showed a similar odds ratio (OR 3.65, 95% CI 1.41-9.49, p<0.01) compared to L3 SM-RA (OR 3.22, 95% CI 1.20-8.61, p=0.02). To evaluate the effect of SM-RA as a continuous variable rather than a binary variable we inserted SM-RA as a continuous variable into our multivariable analysis and observed no change in our conclusion. In both multivariable analyses T4 SM-RA and L3 SM-RA remained significantly associated with postoperative pneumonia OR 0.91(95% CI 0.84-0.99, P=0.02) and OR 0.92 (95% CI 0.87-0.98, P=0.01) respectively.

Figure 3, L3 and T4 multivariable models for the occurrence of postoperative pneumonia and length of hospital stay



Multivariable analysis for hospital admission showed that in both L3 and T4 models, open versus laparoscopic procedure (HR 3.43, 95% CI 2.07-5.70, p<0.01 in both models) and increased age (HR 0.98, 95% CI 0.96-0.99, p=0.01 in both models) were associated with hospital stay. In contrast, L3 and T4 SM-RA were not (HR 1.33, 95% CI 0.94-1.88, p=0.11; HR 1.32, 95%CI 0.94-1.86, p=0.11, respectively) (Figure 3, Appendix 2).

Mortality

Five patients (3%) died within the same admission or within 30 days of surgery. Due to this limited number of events, we could not perform multivariable analysis. We did, however, observe a higher postoperative mortality in patients suffering from postoperative pneumonia (4/21 vs 1/159, p=0.001).

DISCUSSION

Our data suggest that low SM-RA as assessed at both the L3 and T4 level is associated with an increased risk of occurrence of postoperative pneumonia.

The impact of sarcopenia as a risk factor for pulmonary complications has previously been described in studies investigating the association between L3 muscle mass and outcome following esophageal resection (6, 8, 9). Low appendicular muscle mass has also been associated with a higher risk of aspiration pneumonia and 90 day mortality in older patients (7). Moon et al. performed a population wide study in a large cohort of patients aged 65 years and over, and showed that low appendicular muscle mass was independently associated with decreased forced expiratory value and forced vital capacity, which are clinical indicators of pulmonary function (5). Recently, Rozenberg et al. demonstrated that thoracic muscle cross-sectional area was associated with longer hospital stay following lung transplantation (38). In their study, however, cross-sectional thoracic muscle area was not corrected for patient stature, and therefore did not reflect whole body muscle mass. Furthermore, no comparison between thoracic muscle mass and validated L3 measurements for total body muscle mass measurements or SM-RA were performed.

In the clinical setting, CT scans are not performed for the purpose of body composition imaging, but rather to perform tumor staging or assess oncological dissemination. As a consequence, CT scans made in the context of non-abdominal oncological pathologies often do not extend to the L3 level. We therefore argue that T4 SM-RA reflecting thoracic myosteatosis may be a valuable body composition parameter, in particular when no recent abdominal scan is available. The current study therefore holds clinical relevance for cohorts where patients only receive thoracic CT imaging, or in cohorts where only upper abdominal scans are performed, such as in head/neck, lung or upper abdominal cancers like primary liver cancer.

Skeletal muscle radiation attenuation (SM-RA) is a radiological marker of myosteatosis, which is characterised by an increase in intra- and extramyocellular fat deposits, and is highly variable amongst cancer patients (13, 39). Sarcopenia is characterised by a loss of muscle mass. Both sarcopenia and myosteatosis have been found to be independent prognostic factors of reduced survival and poor outcome after surgery or neoadjuvant treatments in various cancer types. A recent study (West MA et al) has shown that reduced SM-RA is associated with a reduction in cardiopulmonary fitness in a HPB population, whereas sarcopenia is not (40). It can therefore be argued that, although sarcopenia and myosteatosis are both associated with poor outcome, they may not be surrogates of the same thing, but rather, that myosteatosis is associated with muscle function, and sarcopenia is associated with muscle mass. This however remains a topic of ongoing research, in which the pathophysiological mechanisms are yet to be clearly defined. In our cohort we found low correlation between SMA and SMRA at the L3 and T4 level ($r=0.21$, $p<0.05$ and $r=0.15$, $p=0.05$) respectively.

The pathophysiological mechanism which may underlie the association between myosteatosis in the thoracic compartment and pneumonia is not clear. It is known that coughing is the most immediate protective mechanism from aspiration, requiring the coordinated activation of inspiratory, expiratory, and intrinsic laryngeal muscles (41, 42). Sillanpaa et al. investigated interdependency between muscle strength and spirometric pulmonary functions in healthy older men and women and observed that reduced muscle strength was associated with reduced pulmonary function (43). It can be hypothesized that a muscle wasting or myosteatosis associated decline of thoracic muscle function reduces this functional protection mechanisms against pneumonia, thus increasing the risk of postoperative pneumonia. Our study suggests that both reduced thoracic and lumbar SM-RA show a significant association with postoperative pneumonia.

Although CT scans were evaluated retrospectively, pneumonia data were collected prospectively by an independent infection registration nurse for quality control purposes. Nevertheless, the use of unvalidated body composition imaging techniques warrants caution and can lead to false premises (44). The limitations of this study must therefore be mentioned. Of the 250 potential cases, 23 were excluded because the thoracic wall was not completely shown. This is a problem seen more often in thoracic scans and related to the fact that thoracic circumference is usually larger than abdominal circumference. Furthermore, subcutaneous thoracic tissue is often segmented out by technicians editing the images because it is traditionally not considered to hold clinical relevance. Our secondary endpoint - length of hospital stay, did not show any association with body composition parameters in multivariable analysis. It must be noted that the retrospectively acquired data regarding discharge generally do not accurately represent the time at which the patient is ready for discharge (45). This means that social or other non-medical reasons for a prolonged hospital stay were not considered in the current study. It must also be noted that the plots showing the correlation between L3 and T4 measurements are notably

scattered (figure 2). This may be caused by biological variation as well as measurement error. In our experience T4 segmentation was slightly more difficult to perform as bone structure needs to be more diligently segmented out of the bodycomposition analysis. We therefore performed an inter-observer analysis which observed similar correlation between L3 and T4 images segmentations. Another explanation for the notable scatter could be a skewed distribution of SM-RA and SMA variables. To better visualise the distribution of T4 and L3 values, we created Z-values by subtracting the mean from each value and dividing it by the standard deviation. Visually, the distribution for SM-RA at L3 and T4 level seem similar. However, L3 measurements were notably skewed (L3 skewness 0.4, T4 Skewness 0.03). To normalize skew, we performed a log normal transformation on L3 and T4 SM-RA, as well as L3 and T4 SMA and found that this did not correct the skew. We subsequently re-plotted the corresponding scatter plots and did not see an improvement in the variation seen in the scatter plots. It may therefore be argued that the scatter seen is indeed a result of biological variation. Another possible reason for variation in measurements could be due to patient-to-patient differences in the axial orientation in the CT scanner, which could lead to variation in segmentation measurements. It is, in the clinical setting, unrealistic to expect an absolutely perfect patient orientation in the CT-scanner. It could be argued that variations in measurements due to imperfect patient orientation may lead to statistical 'noise' in our results. However, we expect variations in measurements due to differences in patient orientation to be minimal.

We neither used previously published cutpoints for SM-RA in our cohort, nor did we create our own stratified cutpoints. In our opinion both the use of previously established cut-offs and establishing of new cut-offs should be approached with caution because it insinuates what should be considered "normal" or "abnormal" bodycomposition values. Body composition varies greatly among regions and ethnicities as illustrated by the large Japanese cohort study of Fujiwara et al., which found highly different cut-offs compared with the study of Martin (e.g. female cut-off for L3-muscle index at 29.6 in the Japanese cohort versus 41 in the Canadian cohort) (26, 33). Further-more optimum stratification for establishing cutoffs works well for large cohorts in which the lowest P-value will be used to set the cut-off (46). However, in smaller cohorts, the P-value is too unstable to use optimum stratification to find a reliable cut-off. We considered our cohort too small for cut-point analysis by optimal stratification, we therefore determined cut-off values for our cohort based on tertiles (46). The discussion regarding the use of cutpoint was brought to life in a recently published editorial in the Journal of Cachexia, Sarcopenia and Muscle, which proposes that unanticipated differences in bodycomposition across international populations also highlight the importance of examining bodycomposition data in the context of the local geographical and ethnic norms, and they strongly support the aim of current multicentre strategies to generate international, disease-specific bodycomposition cutpoints (47).

In conclusion, this study shows that low SM-RA assessed at both the L3 and the T4 level is associated with a higher risk of postoperative pneumonia following CRLM resection. We therefore argue that T4 SM-RA reflecting thoracic myosteatosis may be a valuable body composition parameter, in particular when no recent abdominal scan is available, and thus holds clinical relevance.

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CHAPTER

Is Sarcopenia a Risk Factor for Reduced Diaphragm Function Following Hepatic Resection? A Study Protocol for a Prospective Observational Study

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ABSTRACT

Introduction: Sarcopenia is associated with reduced pulmonary function in healthy adults, as well as with increased risk of pneumonia following abdominal surgery. Consequentially, postoperative pneumonia prolongs hospital admission, and increases in-hospital mortality following a range of surgical interventions. Little is known about the function of the diaphragm in the context of sarcopenia and wasting disorders or how its function is influenced by abdominal surgery. Liver surgery induces reactive pleural effusion in most patients, compromising post-operative pulmonary function. We hypothesize that both major hepatic resection and sarcopenia have a measurable impact on diaphragm function. Furthermore, we hypothesize that sarcopenia is associated with reduced preoperative diaphragm function, and that patients with reduced preoperative diaphragm function show a greater decline and reduced recovery of diaphragm function following major hepatic resection. The primary goal of this study is to evaluate whether sarcopenic patients have a reduced diaphragm function prior to major liver resection compared to non-sarcopenic patients, and to evaluate whether sarcopenic patients show a greater reduction in respiratory muscle function following major liver resection when compared to non-sarcopenic patients.

Methods and analysis: Trans-costal B-mode, M-mode ultrasound and speckle tracking imaging will be used to assess diaphragm function perioperatively in 33 sarcopenic and 33 non-sarcopenic patients undergoing right sided hemi hepatectomy starting one day prior to surgery and up to thirty days after surgery. In addition, rectus abdominis and quadriceps femoris muscles thickness will be measured using ultrasound to measure sarcopenia, and pulmonary function will be measured using a hand-held bedside spirometer. Muscle mass will be determined preoperatively using CT-muscle volumetry of abdominal muscle and adipose tissue at the third lumbar vertebra level (L3). Muscle function will be assessed using handgrip strength and physical condition will be measured with a short physical performance battery (SPPB). A rectus abdominis muscle biopsy will be taken intraoperatively to measure proteolytic and mitochondrial activity as well as inflammation and redox status. Systemic inflammation and sarcopenia biomarkers will be assessed in serum acquired perioperatively.

Ethics and dissemination: This trial is open for recruitment. Is Sarcopenia a Risk Factor for Reduced Diaphragm Function Following Hepatic Resection, a Study Protocol for a Prospective Observational Study is registered at clinicaltrials.gov (EK309-18). The protocol was approved by the official Independent Medical Ethical Committee at Uniklinik (RWTH) Aachen (reference EK 309-18) in July 2019. Results will be published via international peer-reviewed journals and the findings of the study will be communicated using a comprehensive dissemination strategy aimed at healthcare professionals and patients.

Strengths and limitations of this study

- This study prospectively investigates the impact of sarcopenia on diaphragm function of patients undergoing right sided hemi-hepatectomy in a homogenic surgical cohort.
- Patient physical condition and pulmonary function is extensively and objectively recorded.
- This study uses novel non-invasive Speckle Ultrasound to measure diaphragmatic strain as a measure of diaphragm effort.

INTRODUCTION

Sarcopenia and muscle wasting are known risk factors for outcome following hepatic resection [1-5]. Sarcopenia has also been shown to be associated with reduced pulmonary function and increased risk of pneumonia following abdominal surgery [6-10]. Postoperative pneumonia has been shown to prolong hospital admission and increase in-hospital mortality following a range of surgical interventions, including major abdominal and upper gastrointestinal surgery [11-15]. In addition, patients undergoing partial hepatectomy frequently develop reactive pleural effusion and post-operative pneumonia incidences above 10% [11, 13-18]. Besides medical implications, pulmonary complications constitute a significant burden to healthcare systems by increasing healthcare costs [13, 16].

Cachexia and sarcopenia are interrelated wasting disorders, the pathogenesis of which is complex due to its multifactorial nature and is characterized by a negative protein and energy balance that is driven by a variable combination of reduced food intake and abnormal metabolism [19]. The known associations between sarcopenia and pulmonary outcome are based on appendicular muscle mass measurements, or measurements at the third lumbar vertebra (L3). Interestingly, very little is known about the function of the diaphragm in the context of sarcopenia and wasting disorders or how its function is influenced by abdominal surgery [20, 21]. Some preclinical animal studies have shown that sarcopenia is associated with atrophy of diaphragmatic muscle fibers, and that ageing is related to a decline in diaphragmatic function [22-24]. Clinical studies in the context of prolonged mechanical ventilation of acutely ill patients have focused on diaphragm function using ultrasound technology and have shown that prolonged ventilation can lead to diaphragm atrophy which is associated with worse clinical outcome [25-27].

Trans-costal B-mode and M-mode ultrasound is a non-invasive technique which has been used to measure diaphragm function [27-31]. Diaphragm inspirational amplitude (*DIA*) has been shown to decrease significantly following open cholecystectomy [28]. Fractional thickening (*FT*) of the diaphragm has been used in previous studies to quantify effort of the diaphragm [31, 32]. Recently, Deniz et al demonstrated that sarcopenic elderly patients have significantly reduced diaphragm thickness and pulmonary function than non-sarcopenic elderly [21]. Two-dimensional deformation ultrasound or speckle tracking (*ST*) has emerged as a tool which can be used to evaluate diaphragm function in a way that is highly correlated to the gold standard trans diaphragmatic pressure measurements [33, 34]. ST finds its origins in myocardial function ultrasound and enables distinct assessment of cardiac muscle function [34]. The grey value pattern in ultrasound images remains relatively constant for any small region in muscle tissue, this is called a speckle. In the speckle tracking technique, a defined cluster of speckles is tracked from one frame to another during a contractile cycle. This enables the two-dimensional quantification of diaphragm deformation (*strain*).

We hypothesize that both major hepatic resection and sarcopenia have a measurable impact on diaphragm function. Furthermore, we hypothesize that sarcopenia is associated with reduced preoperative diaphragm function, and that patients with reduced preoperative diaphragm function show a greater decline and reduced recovery of diaphragm function following right sided hemi hepatectomy.

The primary goal of this study is to evaluate whether sarcopenic patients have a reduced diaphragm function prior to major liver resection compared to non-sarcopenic patients, and to evaluate whether sarcopenic patients show a greater reduction in respiratory muscle function following right sided hemi hepatectomy when compared to non-sarcopenic patients. The secondary objective of this study is to investigate proteolytic activation and markers of mitochondrial activity in muscle tissue, as well as systemic inflammation markers of sarcopenic and non-sarcopenic patients.

METHODS & ANALYSIS

In- and exclusion criteria

This study will entail a prospective observational single center study, analyzing consecutive patients undergoing open right sided hemi hepatectomy with biliary reconstruction between the ages of 18- and 80 years. Surgery is performed as part of standard care. Only patients undergoing open right sided hemi hepatectomy with biliary reconstruction will be included in the study to achieve a homogenous study cohort. Exclusion will be on the basis of American Anesthesiology Association (ASA)-classification IV or higher, Liver cirrhosis Child grade B or higher, end stage renal disease requiring dialysis, severe heart disease New York Heart Association class IV, preexisting pulmonary conditions including, chronic obstructive pulmonary disease (COPD), asthma, history of pulmonary surgery, history of pulmonary embolism, smoking, pleural effusion occupying more than 1/3 of the pleural space, neurological disorders leading to paraparesis of the upper or lower limbs, or known muscular dystrophic disorders. Patients will consecutively be assigned to a sarcopenic and non-sarcopenic group based on CT-muscle quantification and hand grip strength (definitions will be detailed below). Sarcopenia stratification will be performed by an investigator blinded for the diaphragm ultrasound and pulmonary function testing and vice versa.

Patients will receive postoperative analgesia in the form of standard postoperative analgesia protocols. Administration of analgesia and pain scoring (Visual Analog Scales) will be recorded by the primary investigator to correct for pain-associated restriction of pulmonary function.

Blinding of results

To ensure unbiased ultrasound evaluation and pulmonary function testing, the investigator performing diaphragm ultrasound and pulmonary function testing will be

blinded for the sarcopenia stratification. Thus, investigator 1 will perform stratification of patients into sarcopenic and non-sarcopenic groups based on L3 CT and handgrip strength measurements. Investigator 2 will perform ultrasound and pulmonary function testing as well as physical condition testing and blood sampling.

Endpoints

Primary endpoints

Differences in diaphragm kinetics as measured by ST ultrasound (e.g. deformation% (strain), deformation velocity (strain rate), and dimensional measures which are fractional thickening and range of DIA) and abdominal muscle kinetics (e.g. deformation%, deformation velocity, fractional thickening, and DIA, see above) between sarcopenic and non-sarcopenic study groups prior to elective right sided hemi hepatectomy.

Secondary endpoints

Longitudinal changes from baseline values in diaphragm and abdominal muscle (rectus abdominis muscle) kinetics (e.g. deformation%, deformation velocity, fractional thickening and DIA), and the occurrence and quantification of pleural effusion in the postoperative phase across sarcopenic and non-sarcopenic groups following elective right sided hemi hepatectomy as measured by ST ultrasound compared to each other and pre-operative values.

Tertiary endpoints

Perioperative quantitative and qualitative evaluation of a panel of biomarkers associated with inflammation, mitochondrial function and the pathogenesis of sarcopenia (see appendix 1) in the sarcopenic and non-sarcopenic groups.

Defining sarcopenia & physical condition

Sarcopenia will be defined as either low estimated muscle mass measured by CT-muscle volumetry or reduced muscle function measured by handgrip strength, or reduced physical condition as defined by the European Working Group on Sarcopenia in Older People (EWGSOP) [35].

CT muscle quantification

CT-muscle volumetry of abdominal muscle and adipose tissue at L3 will be performed on abdominal CT-scans. This method provides an accurate estimation of whole-body muscle mass as well as the detection of sarcopenia [36]. These scans will be taken for routine diagnostic purposes and will not lead to additional radiation exposure of patients for the purpose of this study. Muscle volumetry at the L3 level will be corrected for patient stature. Using gender and BMI specific cut offs as described by Martin et al. (L3-skeletal muscle

index of <55 cm^2/m^2 for men or <39 cm^2/m^2 for women), patients will be categorized as sarcopenic or non-sarcopenic [36, 37]. In addition, other body composition parameters, such as muscle radiation attenuation, visceral fat mass, and subcutaneous fat mass will be evaluated for association with our primary outcome variable.

Functional muscle measurement (handgrip strength)
In addition to muscle mass, a measurement of muscle function will be performed. A Handgrip strength test will be performed to ascertain muscle strength on both hands with the elbow flexed at 90°. Three repeats will be performed and the highest value will be used for analysis. The value of the dominant hand will be used for stratification into sarcopenic and non- sarcopenic groups. This method has been validated for the detection of sarcopenic patients and cut off points for detection have been defined by the European Working Group on Sarcopenia in Older People (EWGSOP) [35].

Physical condition test
To assess physical performance, a Short Physical Performance Battery (SPPB) test will be performed. The SPPB has been recently recommended by an international working group for use as a functional outcome measure in clinical trials in frail older persons and is recommended as part of the detection algorithm for sarcopenia by the EWGSOP [38]. Furthermore, cut off points have been defined by the EWGSOP.

Ultrasound
Patients will undergo a transcostal ultrasound in the supine position one day prior to surgery, as well as on the first-, third-, fifth-, and seventh postoperative day. The ultrasound transducer will be positioned longitudinally to the anterior axillary line between the 9^{th}- and 11^{th} intercostal space. In this location, the diaphragm is identified as a three-layered structure just superficial to the liver, consisting of a relatively non-echogenic muscular layer bounded by the echogenic membranes of the diaphragmatic pleura and peritoneum. A ten second recording will be made at maximum frame rate for analysis. In addition, pleural effusion will be observed, and if present, quantified.

B-mode and M-mode Ultrasound Fractional thickening (FT) will be measured using a 13-MHz linear array transducer. Diaphragmatic thickness will be measured at end-expiration ($T_{di,ee}$) and peak inspiration ($T_{di,pi}$; i.e. peak thickness value during inspiration) as the distance between the diaphragmatic pleura and the peritoneum using M-mode. Measurements of $T_{di,ee}$ and $T_{di,pi}$ will always be made on two respiratory cycles visualized in a single M-mode. Diaphragm thickening during inspiration (DT_{di}) will be taken as the difference between $T_{di,pi}$ and $T_{di,ee}$. Diaphragm thickening fraction (TF_{di}) will be defined as the percentage change in diaphragm thickness during inspiration (computed from the quotient of DT_{di} and $T_{di,ee}$) [31, 32]. *Diaphragm inspirational amplitude* (*DIA)* will be determined using M-mode ultrasound [27].

Speckle tracking (ST) analysis

Following the ultrasound recording of diaphragm kinetics, images will be analyzed using software to ascertain strain rate, fractional thickening, and range of motion.

Strain describes the relative change in length between an initial reference state (L0) and compressed/shortened state (L). The conventional strain is defined as: $\varepsilon = (L-L0)/L0$. Positive strain means stretching, whereas negative strain means shortening. To investigate strain, a region of interest (ROI) will be placed between the echogenic line of the peritoneum and pleural line. Strain rate indicates the rate of deformation as follows: $\varepsilon' = d\varepsilon/dt$. Strain rate is an instantaneous measurement not requiring a relation to a reference state. The strain will be measured as longitundinal strain using commercially available software (EchoPac®, GE Healthcare)

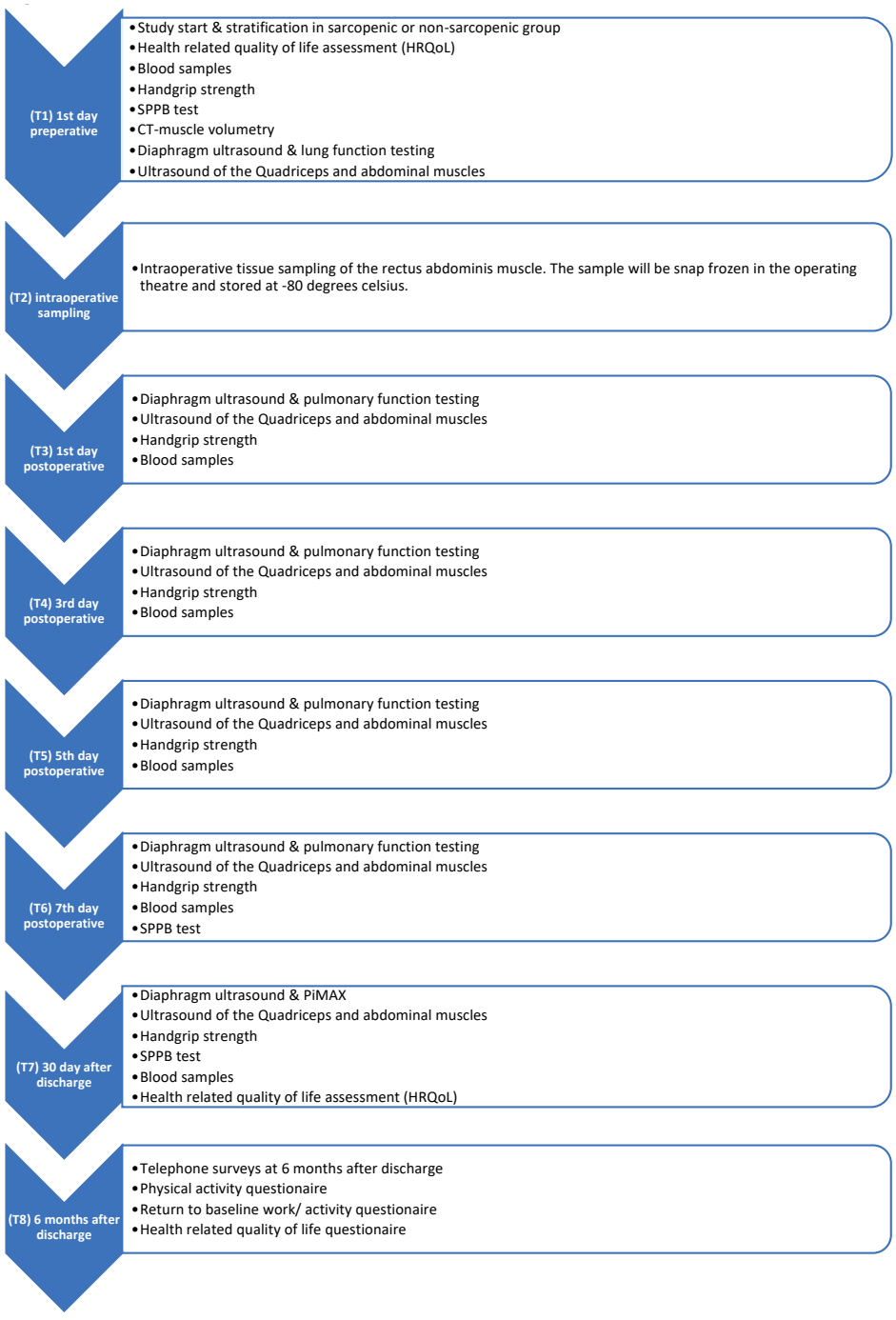
Lung function testing

Following ultrasound, diaphragm kinetics will be recorded during normal breathing on both sides, and once at pi-max (peak inspiratory pressure). Pi-max will be measured with a portable hand-held spirometer (Micro RPM handheld spirometer) which patients must breath through at maximum capacity. This will provide broad insight into the general respiratory capacity, as well as at maximum effort.

Study time plan

Patients will be asked to participate in the study at the time of first contact in the outpatient clinic, in the weeks prior to surgery. Data collection will commence one day prior to surgery and will end 6 months after discharge from the hospital. Data will be collected at eight timepoints (T1 though T8) during this period, namely one day prior to surgery, during surgery, and at day 1, 3, 5, and 7 postoperatively. Thirty days after surgery, data collection will be performed during a routine postoperative checkup. Six-months after surgery, a telephone questionnaire focused on physical activity and quality of life (36-Item Short Form Health Survey Questionnaire, SF36) will be performed (see study flowchart figure 1). Drop-outs from the study as well as loss to follow up and missing data will be recorded and stated in the final draft of the manuscript.

Figure 1. Study time plan.



Flowchart showing study time plan from T1 one day preoperative to T8 6 months after discharge.

- *T1 (one day preoperative)*
  First data collection. Patients will be stratified as being sarcopenic or non-sarcopenic. An additional blood sample will be taken and frozen (5ml EDTA) from the antecubital vein. The Handgrip strength test will be performed to assess muscle strength. The Short Physical Performance Battery (SPPB) test will be performed to determine physical condition (appendix 2). CT-muscle volumetry will be performed on existing preoperative CT-scans to distinguish sarcopenic and non-sarcopenic patients and assignment to sarcopenic and non-sarcopenic groups. Diaphragm, quadriceps femoris, and rectus abdominis ultrasound will be performed as previously described. Lung function testing will be performed at the bedside as described. A Health-related quality of life questionnaire will be filled out (HRQoL)(36-Item Short Form Health Survey Questionnaire, SF36).

- *T2 (intraoperative sampling)*
  Intraoperative tissue sampling of the m. rectus abdominis, samples will be snap frozen in liquid nitrogen in the operating theatre and stored at -80 degrees Celsius. Sample size will be approximately 1cm^3 and will be resected using non-electric scissors.

- *T3 (day 1 postoperative)*
  Diaphragm, quadriceps femoris and rectus abdominis ultrasound will be performed. Handgrip strength test will be performed, and blood samples will be taken from the antecubital vein; serum or plasma will be processed and frozen.

- *T4 (day 3 postoperative)*
  Diaphragm, quadriceps, and rectus abdominis ultrasound will be performed. Handgrip strength test will be performed, and blood samples will be taken; serum or plasma will be processed and frozen.

- *T5 (day 5 postoperative)*
  Diaphragm, quadriceps, and rectus abdominis ultrasound will be performed. Handgrip strength test will be performed, and blood samples will be taken; serum or plasma will be processed and frozen.

- *T6 (day 7 postoperative)*
  Diaphragm, quadriceps, and rectus abdominis ultrasound will be performed. Handgrip strength test will be performed and blood samples will be taken; serum or plasma will be processed and frozen. Health related quality of life questionnaire will be filled out (HRQoL). The SPPB test will be performed.

- *T7 (30 days after hospital discharge)*
  Diaphragm, quadriceps, and rectus abdominis ultrasound will be performed. Handgrip strength test will be performed, and blood samples will be taken; serum or plasma will

be processed and frozen. Health related quality of life questionnaire will be filled out (HRQoL). The SPPB test will be performed.

- *T8 (6 months after surgery)*
  A telephone questionnaire will be performed containing a physical activity questionnaire, return to baseline work/activity questionnaire and a health-related quality of life questionnaire (36-Item Short Form Health Survey Questionnaire, SF36).

Setting
Recruitment of patients and subsequent sampling will be performed tertiary university hospital in Germany, Uniklinik RWTH Aachen.

Statistical analysis
Power analyses
No data regarding speckle imaging variables (strain or strain rate) amongst different patient groups are available. However, Deniz et al. recently investigated differences in lung function between older sarcopenic and non-sarcopenic patients by measuring diaphragm thickness [21]. We calculated the expected effect size based on mean diaphragm thickness values and standard deviation (SD) after forced expiration of the aforementioned study. Based on a mean FT in the non-sarcopenic group (n=30) of 1.5mm (SD 0.7) and 1.1mm (SD 0.4) in the sarcopenic group (n=30), we calculated an effect size of 0.70. Considering an alpha of 0.05 and a beta of 0.80, a total sample size of 66 patients (33 patients per group) will be needed.

Analysis of Primary and Secondary Outcome Parameters
SPSS will be used for statistical analysis (IBM Corp, Released 2013, IBM SPSS Statistics for Macintosh, Version 22.0.). A 2-tailed *P* value <.05 will be considered statistically significant. To allow comparisons between groups, data will be tested for normal distribution, and appropriate statistical tests will be applied, potentially including Students *t* test, Mann-Whitney U test, analysis of variance, Kruskal-Wallis test, Chi-square test, or Fisher exact test. Normally distributed continuous variables will be evaluated as mean values and standard deviation. Non-normally distributed continuous variables will be evaluated as median and range.

Patient and public involvement
No patients involved

Ethics and dissemination
Diaphragm Kinetics following Hepatic Resection, comparison between a sarcopenic and non-sarcopenic cohort is registered at clinicaltrials.gov (EK309-18). The protocol was ethically approved by the official Independent Medical Ethical Committee of the

Uniklinik (RWTH) Aachen (reference EK 309-18) in July 2019. Written informed consent will be obtained from all participants. The study will be performed in accordance with the principles of the Declaration of Helsinki, as well as the guidelines of Good Clinical Practice. Recruitment started in the first quarter of 2020, and recruitment is currently ongoing. Patients deemed eligible for enrollment are initially recruited by their surgeon at the time of approval for surgery. If interested in participation, the patient will be contacted by the researcher and given detailed information about the study, in both oral and written form. After a 2-week period, the subjects are contacted to obtain informed consent, and then they will be officially enrolled in the study.

DISCUSSION

Sarcopenia is characterized by concurrent hypermetabolism, hypercatabolism and hypoanabolism which aggravate weight loss and are provoked by tumour induced systemic inflammation and catabolic factors party mediated by the central nervous system [39]. Irrespective of the cause, sarcopenia has been shown to have a significant negative impact on short- and long-term outcome following a range of oncological treatments [1, 3, 37, 40-45]. Not only long-term outcome has been shown to be impacted by sarcopenia. Postoperative complications (morbidity) have profound impact on the burden of disease and suffering following surgical treatment [4, 41, 42, 46-48]. Pulmonary morbidity, especially postoperative pneumonia, is of particular importance and has been shown to prolong hospital admission and increase in-hospital mortality following a range of surgical interventions, most notably after major abdominal and upper gastro-intestinal surgery [11-15].

The results of this study will contribute to the understanding of the role of the diaphragm in pulmonary morbidity following liver resection and will provide insight into the role of sarcopenia in pulmonary morbidity. To our knowledge, this approach has not previously been implemented for the investigation of the impact of abdominal surgery or sarcopenia and wasting disorders on diaphragm function.

We hypothesise that the results of this study will contribute to identifying patients at risk of pulmonary complications following liver resection. Identification of these at-risk patients is of great importance for the implementation of improved preconditioning of patients, thus potentially contributing to the reduction of postoperative pulmonary morbidity in the future.

"Is Sarcopenia a Risk Factor for Reduced Diaphragm Function Following Hepatic Resection, a Study Protocol for a Prospective Observational Study", is a study currently recruiting patients.

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CHAPTER

Identifying Radiomics Signatures in Body Composition Imaging for the Prediction of Outcome Following Pancreatic Cancer Resection

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ABSTRACT

Background: Computerized radiological image analysis (radiomics) enables the investigation of image-derived phenotypes by extracting large numbers of quantitative features. We hypothesized that radiomics features may contain prognostic information that enhances conventional body composition analysis. We aimed to investigate whether body composition-associated radiomics features hold additional value over conventional body composition analysis and clinical patient characteristics used to predict survival of pancreatic ductal adenocarcinoma (PDAC) patients.

Methods: Computed tomography images of 304 patients undergoing elective pancreatic cancer resection were analysed. 2D radiomics features were extracted from skeletal muscle and subcutaneous and visceral adipose tissue (SAT and VAT) compartments from a single slice at the third lumbar vertebra. The study population was randomly split (80:20) into training and holdout subsets. Feature ranking with Least Absolute Shrinkage Selection Operator (LASSO) followed by multivariable stepwise Cox regression in 1000 bootstrapped re-samples of the training data was performed and tested on the holdout data. The fitted regression predictors were used as "scores" for a clinical (C-Score), body composition (B-Score), and radiomics (R-Score) model. To stratify patients into the highest 25% and lowest 25% risk of mortality compared to the middle 50%, the Harrell Concordance Index was used.

Results: Based on LASSO and stepwise cox regression for overall survival, ASA ≥3 and age were the most important clinical variables and constituted the C-score, and VAT-index (VATI) was the most important body composition variable and constituted the B-score. Three radiomics features (SATI_original_shape2D_Perimeter, VATI_original_glszm_SmallAreaEmphasis, and VATI_original_firstorder_Maximum) emerged as the most frequent set of features and yielded an R-Score. Of the mean concordance indices of C-, B-, and R-scores, R-score performed best (0.61, 95% CI 0.56–0.65, $p<0.001$), followed by the C-score (0.59, 95% CI 0.55-0.63, $p<0.001$) and B-score (0.55, 95% CI 0.50–0.60, $p=0.03$). Kaplan-Meier projection revealed that C-, B, and R-scores showed a clear split in the survival curves in the training set, although none remained significant in the holdout set.

Conclusion: It is feasible to implement a data-driven radiomics approach to body composition imaging. Radiomics features provided improved predictive performance compared to conventional body composition variables for the prediction of overall survival of PDAC patients undergoing primary resection.

INTRODUCTION

The impact of cachexia, sarcopenia and myosteatosis on outcome following oncological surgery has been widely established [1-3]. Reduced muscle function has been shown to be associated with myosteatosis, which is defined as increased inter- and intramyocellular fat stores[4, 5]. It can be quantified by assessing skeletal muscle radiation attenuation (SM-RA) on Computed Tomography (CT)-scans [6-8]. Both myosteatosis and high visceral adiposity have been reported to be associated with worse overall survival following a pancreatic oncological resection of Pancreatic ductal adenocarcinoma (PDAC) [9]. Surgical resection is the only curative therapy available for the treatment of PDAC, but due to loco-regional advancement or metastasis, only 20% of pancreatic carcinomas are treatable by resection [10]. Despite efforts to improve treatment efficacy, overall short- and long-term survival rates have remained poor over the past decades [11]. This is partly due to wasting conditions such as cachexia and sarcopenia which occur in the vast majority of patients with pancreatic cancer [12, 13]. Identifying patients who are predisposed to respond badly to surgical treatment based on body composition is of added value for personalized treatment strategies or pursuing non-surgical treatment alternatives.

Advances in CT image analysis have enabled identification of 'tumor phenotypes' by extracting large numbers of quantitative features from radiological images, i.e. the field currently known as radiomics. Radiomics features have been shown to provide prognostic value in predicting clinical outcomes of several tumor entities, including head and neck cancer and lung tumors [14-17]. However, a recently published study of non-small cell lung carcinoma (NSCLC) patients undergoing chemotherapy treatment was unable to identify radiomics features that predict muscle loss [18]. That study only investigated radiomics features of muscle tissue for the prediction of muscle loss. Chen et al, recently implemented a radiomics approach to the identification of sarcopenia in gastric cancer patients, and showed that radiomics measured sarcopenia outperformed conventional body composition analysis for survival and complication prediction [19]. We hypothesized that additional phenotypic information related to body composition can be extracted from muscle, subcutaneous fat, and visceral fat compartments by a radiomics-based analysis of CT images in PDAC patients.

Our goal was to investigate whether radiomics-based body composition features can discriminate between patient groups with increased or decreased overall survival following curative resection for the treatment of PDAC. In addition, the performance of radiomics-based body composition analysis for the stratification of short versus long overall survival was compared to body composition variables obtained by conventional manual CT-scan analysis and established clinical patient characteristics.

METHODS

Patients

All patients that had a resectable PDAC of the pancreatic head and were treated at Uniklinik Aachen (UKA) or Maastricht University Medical Center (MUMC), between 2010 and 2017 were eligible for inclusion. Patients were excluded from analysis on the basis of American Society of Anesthesiology (ASA) classification V, (severe liver cirrhosis with Child grade C, end-stage renal disease requiring dialysis, severe heart disease), New York Heart Association class IV, and/or chronic obstructive pulmonary disease (COPD) requiring (home)oxygen therapy and administration of neoadjuvant treatment. In addition, patients were excluded if CT-scans did not include the abdominal wall or when the interval between the time of the scan and surgery was greater than three months. Besides body composition, we evaluated age at the time of surgery and ASA-classification and BMI as clinical predictors [23, 24]. Clinical data was acquired from a prospectively acquired database and retrospectively analysed. Ethical approval was obtained prior to this study from the local medical ethical board.

CT body composition variables

Body composition was analysed using electronically stored venous or porto-venous intravenous contrast phase of abdominal CT-scans acquired during routine clinical practice. CT-scans were selected and analysed while blinded to the mortality outcomes by an experienced single investigator, trained using the gold standard of bodycomposition imaging methodology as described by Prado and Baracos et al, using Slice-O-matic software, version 5.0 (Tomovision, Montreal, QC, Canada) [20].

An overview of the different scanner parameters can be found in Appendix 1 of the supplemental material.

The third lumbar vertebra (L3) was used as a standard landmark to measure tissue cross-sectional area in cm^2 as previously reported [13]. In short, skeletal muscle area (SMA), visceral adipose tissue (VAT) area, and subcutaneous adipose tissue (SAT) area were quantified on CT images with manual segmentation using predefined Hounsfield Unit (HU) ranges (SM: -29 to 150 HU, VAT: -150 to -50 HU, and SAT: -190 to -30 HU) [25]. SM, VAT, and SAT were corrected for stature to calculate the skeletal muscle index (SMI), VAT-index (VATI), and SAT-index (SATI) in cm^2/m^2, providing good estimates of total body SM, VAT, and SAT mass [25]. Skeletal muscle radiation attenuation (SMRA) was assessed by calculating the average HU value of the total muscle area within the specified range of -29 to 150 HU.

Body composition greatly varies with gender. SM, VAT, SAT, SM-RA were therefore expressed as Z-scores. The Z-score is defined as the number of standard deviations each patient differs from the mean value of patients belonging to the same sex. The use of

Z-scores facilitates comparison of the effects of body composition in heterogeneous patient cohorts, normalizing for the sex-based differences.

Defining endpoints

The main clinical endpoint for the evaluation of survival following surgery was overall survival with a follow up of 5 years following surgery. In order to stratify patients into the best and worst performing 25% regarding overall survival compared to the middle 50%, Harrell Concordance Indexes (c-index) was used as the statistical discrimination metric. A body composition radiomics model was designed to evaluate whether bodycomposition radiomics features could predict best, middle and worst performing patients regarding overall survival, i.e. low, middle and high risk patients following pancreatic resection.

Radiomics features optimization and model building

In each segmented body composition region (SMA, VAT, and SAT), 114 individual radiomics features (342 in total) were automatically extracted using the open-source software library PyRadiomics 2.0.1 [26]. The images were interpolated to a fixed 2mm grid during feature extraction to reduce unwanted variation. No digital image pre-processing filter was used. The model-building process consisted of the following key steps (illustrated schematically in Figure 1):

Step 1: Training and holdout validation subsets

The study population was split 80:20 into training and holdout sets, respectively, each maintaining the same proportion of Aachen and Maastricht patients as in the whole population. Survival outcome was calculated as time interval to death from date of surgery.

Step 2: Feature value transformation

A Yeo-Johnson transformation, followed by centering to a mean of zero and scaling to a standard deviation of one, was used to correct for highly skewed distributions of body composition and radiomics features in the training set [21]. These same transformations, without recomputation, were applied directly on the holdout set.

Step 3: Radiomics feature selection through regularization

We first created 1000 random bootstrap samples (i.e. resampling from the training set with replacement) of the same size as the training set. These same 1000 resamples were kept fixed for feature selection (FS) and model development.

FS was applied only on the 342 radiomics features. For the clinical and body composition models, FS was omitted because these feature sets were already quite small (containing 3 and 4 predictors respectively as described in step 4).

In each bootstrap FS sample, we first calculated the radiomics features with very high pair-wise Spearman correlations (> 0.90). A recursive elimination algorithm was then used to remove the maximum number of redundant features. A 5-fold internally cross-validated LASSO (Least Absolute Shrinkage and Selection Operator) Cox regression was applied to the remaining features. The LASSO-selected individual features from each of the 1000 bootstrapped FS samples were pooled and ranked (from high to low) by its frequency of selection. We called these "surviving" features the *"feature pool"*. Since there is currently no universally accepted threshold for how frequently features ought to appear in a pool, we arbitrarily chose a cut-off frequency of 500. Features appearing less than 500 times out of 1000 bootstrap samples were assumed to be too sensitive to sampling and were therefore excluded from the stepwise regression step.

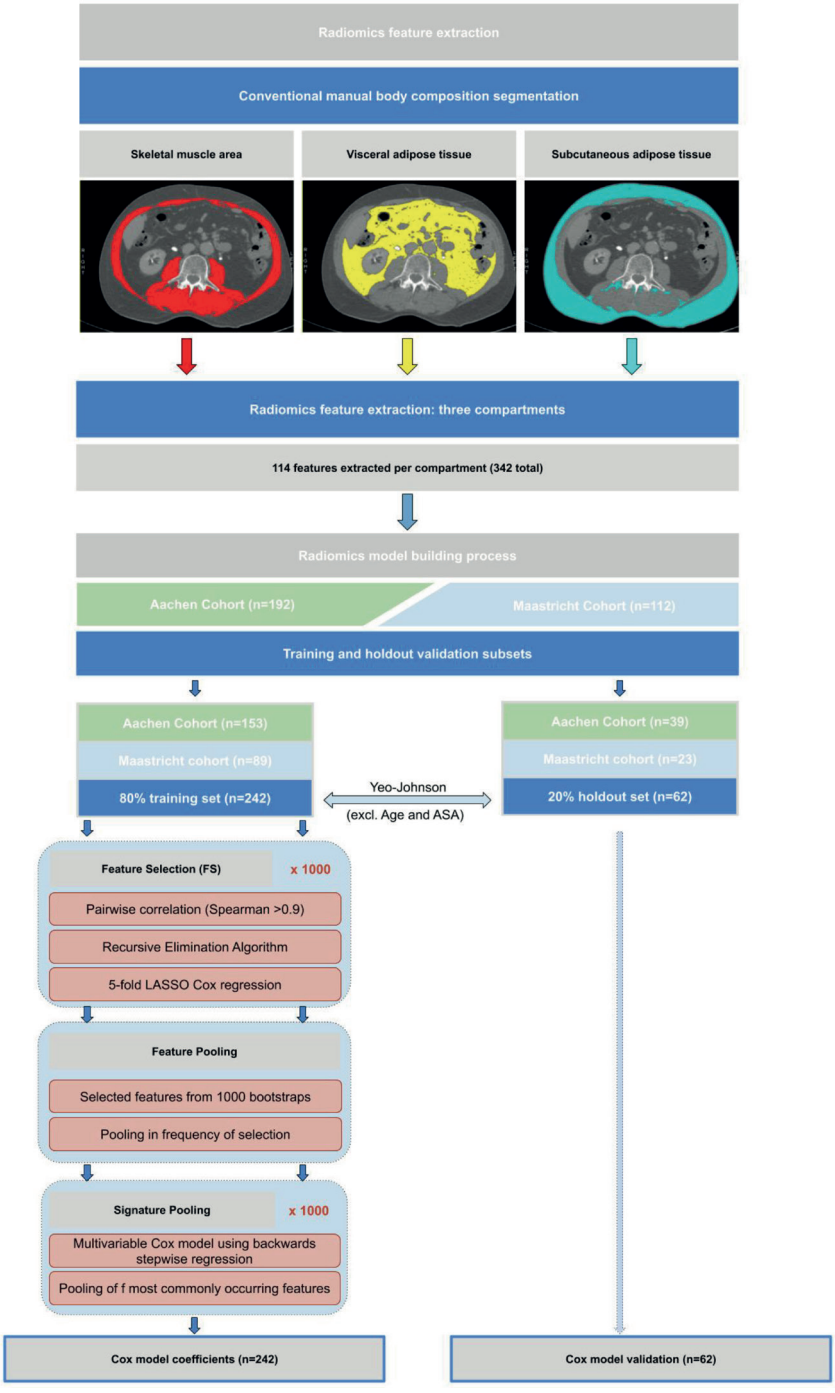
Step 4: Signature pooling with stepwise Cox regression

The same 1000 subsamples as described above were used to assemble a multivariable Cox model using backwards stepwise regression, with the objective to minimize the Akaike Information Criterion [22]. This has the effect of testing different combinations of the most frequently LASSO-retained radiomics features. As before, we summed up the selected frequency of sets of features that appeared together, which we called the *"signature pool"*. Given that there is no consensus regarding which signature to choose from a number of alternatives, we decided to take the most-frequently appearing combination of features from the signature pool. The same process was used to build the clinical and body composition signatures. The clinical features selected by clinical experience *a priori* were – Age, BMI, ASA ≥3 and sex. The body composition features selected *a priori* were – SM-RA, SMI, VATI and SATI.

Statistical analysis

The above mentioned feature selection and statistical analysis for survival was performed in R [23]. Following Altman et al. [24], we computed a linear predictor, or "score", from the sum of products of each feature with its coefficient. We computed the Harrell Concordance Index (C-index) for the training and holdout sets [25]. The fitted regression predictors were used as "scores" for a clinical (C-Score), body composition (B-Score), and radiomics (R-Score) model. In order to stratify patients into the best and worst performing 25% regarding overall survival compared to the middle 50%, Harrell Concordance Indexes (c-index) was used as the statistical discrimination metric.

Figure 1. Model Building



Flowchart showing methodological approach to radiomics feature extraction from CT scans, as well as the statistical model building methodology approach as described in the model building section.

RESULTS

Patient characteristics

Of the 425 patients included in the cohort, body composition analysis could be performed for 304 patients. In 52 cases, there was missing survival data, 14 patients were excluded on the basis of poor-quality CT scans, 25 were excluded due to scans not showing the abdominal wall, and 30 patients were excluded due to an interval greater than three months between the time of the scan and surgery. 53% of patients were male, with a mean age of 67.7 (SD 10.2) and mean BMI of 25.4 kg/m^2 (SD 4.2). 42% of patients had a high ASA-score (≥3). 91.7% of patients underwent a Pylorus-Preserving Pancreatico Duodenectomy (PPPD) and 8.3% underwent a Whipple procedure (the same operation without preservation of the pylorus). Only PDAC cases (ductal adenocarcinoma) were included in the study. Ninety-day and two-year mortality rates were 10% and 45%, respectively. No differences were observed between in- and excluded cases regarding sex, age, ASA-score, or TNM classification (all p>0.10). Patient demographics across the training and validation cohorts are shown in table 1.

Table 1. Study demographics

		Training-set	Validation-set	p-value
Age (mean)		66	68	0.21
Sex				
	Male	114	28	0.86
	female	132	31	
BMI (mean)		25.6	25.9	0.48
ASA (mean)		2.41	2.47	0.39
Tumor stage				0.47
	1A	3	1	
	1B	12	1	
	2A	46	17	
	2B	161	36	
	3	23	4	
SMI (mean)		44.7	46.9	0.90
SM-RA (mean)		34.4	34.2	0.91

Demographics of relevant study parameters across training- and validation-cohorts. P-value calculated using independent T-test for continuous variables and Chi-square test for binary variables.

Feature selection and pooling results

Model building from feature selection to signature pooling (steps 1 to 4) was performed (see Figure 1) to select the most significant clinical-, body composition- and radiomics features and yielded a clinical score (C-score), body composition score (B-score), and a radiomics score (R-score).

C-score

Age at surgery in years, ASA ≥ 3 and sex were used as prognostic clinical features for overall survival. The linear predictor equation in the Cox regression model was:

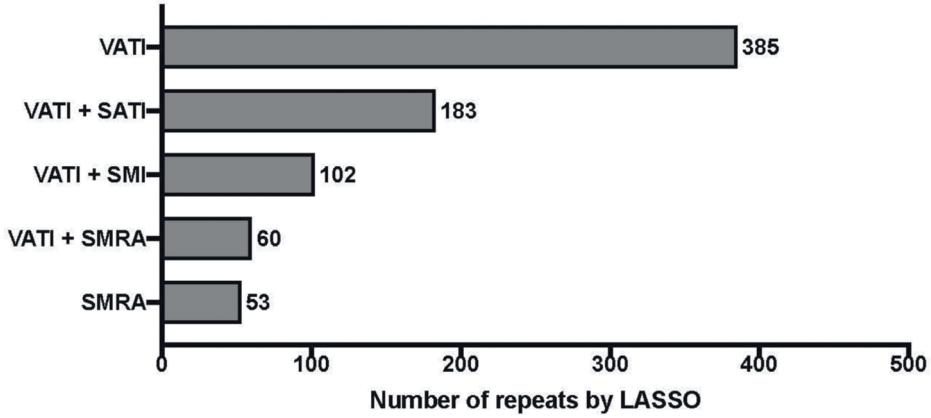
C-Score = 0.01353*(Age in years) + 0.4087*(ASA ≥ 3) + 0.2803*(sex; male = 1) (Equation 1)

B-score

The relative importance of the body composition variables SMI, SM-RA, VATI, and SATI for survival is shown in Figure 2. Increased VATI emerged as prognostic clinical feature for overall survival. The Cox model linear predictor was:

B-Score = 0.1988*(VATI) (Equation 2)

Figure 2. Frequency table showing the relative importance of body composition variables for overall survival.



Relative importance of body composition variables of CT-scans on overall survival. "least absolute shrinkage and selection operator" (LASSO) penalized Cox regression with 5-fold cross-validation. Bootstrap resampling with univariate rejection and LASSO was repeated for a total of 1000 unique random number generator seeds. The frequency table, i.e. "feature pool", of each body composition variable is shown: Visceral Adipose Tissue Index (VATI), Skeletal Muscle Radiation Attenuation (SM-RA), Skeletal Muscle Index (SMI), Suboutaneous Adipose Tissue Index (SATI).

R-score

The results of *feature pooling* and *signature pooling* of the radiomics feature selection are shown in Figure 3 and Figure 4, respectively. Four candidate radiomics features showed up more than 500 times and were selected from the LASSO-based feature pool. Various combinations of these four features were tested in the *signature pooling* step, and the most frequent set of features yielded an R-Score linear predictor comprising only 3 radiomics features:

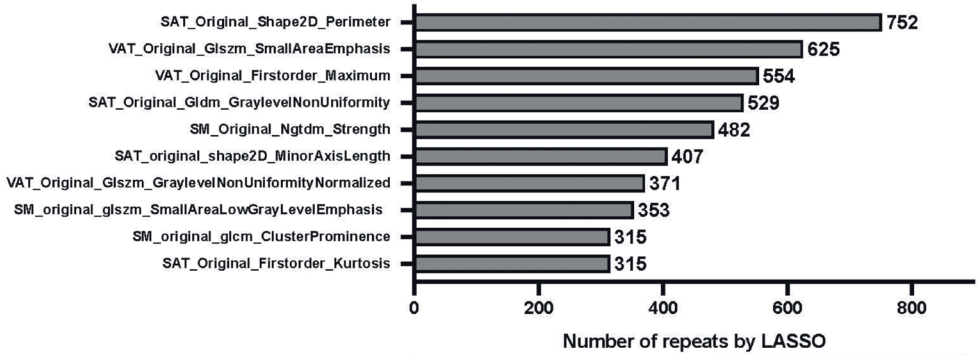
R-Score =

0.3100*(SATI_original_shape2D_Perimeter)

- 0.2302*(VATI_original_glszm_SmallAreaEmphasis)

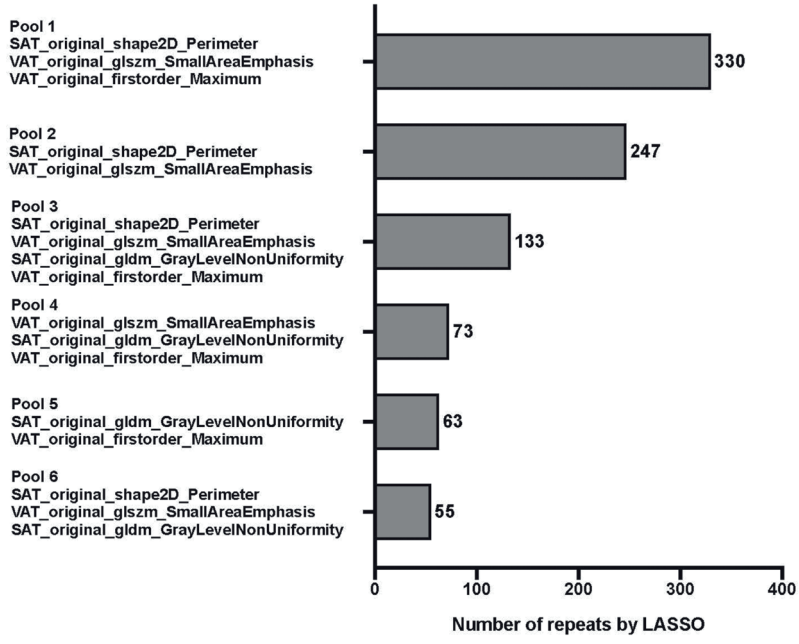
+ 0.1353*(VATI_original_firstorder_Maximum) (Equation 3)

Figure 3. Relative importance of radiomics features for overall survival.



"Least absolute shrinkage and selection operator" (LASSO) penalized Cox regression with 5 fold crose-validation. Bootstrap resampling with univariate rejection and LASSO was repeated for a total of 1,000 unique random number generator seeds. The figure shows the frequency table of every radiomic feature that had a non-zero coefficient., with SAT_original_shape2D_Perimeter showing highest number of repeats, i.e. being the most important radiomics feature. "VAT", "SAT", and "SM" in the formula refer to radiomics features extracted from visceral adipose tissue, subcutaneous adipose tissue, and skeletal muscle, respectively.

Figure 4. Selection Frequency of Pooled Radiomics Features.



Features were pooled by using 1,000 unique bootstrap samples consisting of a random 75% of the FS subset into a backwards stepwise Cox regression against survival time to assemble individual features into predictive signatures. The stepwise elimination criterion was the Akaike Information Criterion [1]. Frequency tables were compiled for every unique combination of features, ie signature The pool with the highest number of repeats (pool 1) was finally selected for further modeling.

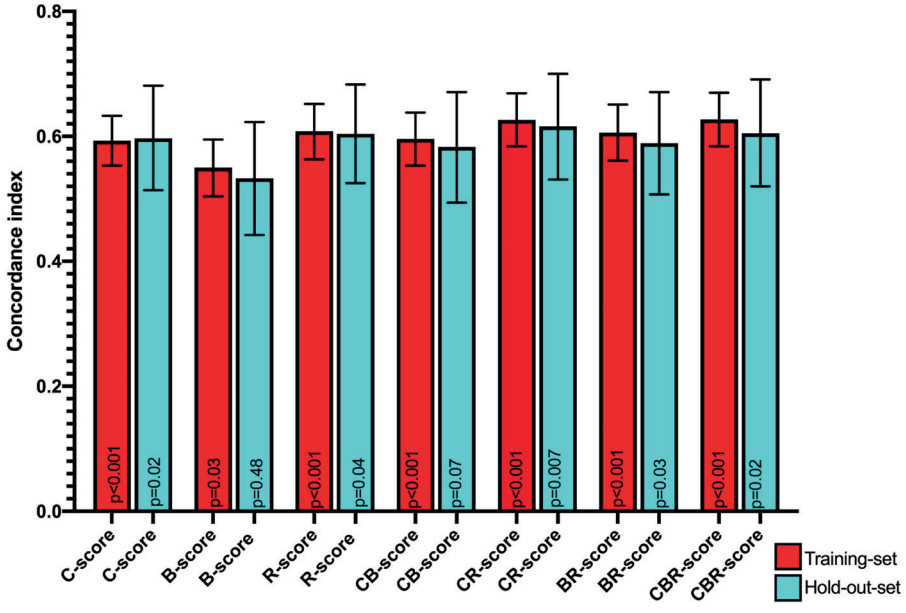
To evaluate whether combined clinical, bodycomposition and radiomics models might outperform individual scores, we created combined CR (clinical & radiomics), CB (clinical and bodycomposition) and CBR (clinical, body composition and radiomics) scores.

Modelling results for overall survival

In the training set, the mean Harrell C-indices for the overall survival time model were highest for the R-score (0.61, 95% CI 0.56 – 0.65, p<0.001), followed by the C-score (0.59, 95% CI 0.55 - 0.63, p<0.001) and B-score (0.55, 95% CI 0.50 – 0.60, p=0.03).

All three concordance indices were comparable in the holdout set compared to the training set: R-score: 0.60, 95% CI 0.53 – 0.68, p=0.04, C-score: 0.60, 95% CI 0.51 - 0.68, p=0.02, and B-score: 0.53, 95% CI 0.44 – 0.62, p=0.48, with the B-score not retaining significance in the hold out set (Table 1, Figure 5). Combined CR-score: 0.63, 95% CI 0.58 – 0.67, p<0.001, CB-score: 0.60, 95% CI 0.55 – 0.64, p<0.001 and CBR-score: 0.63, 95% CI 0.58 – 0.70, p<0.001, showed similar predictive value as individual scores, which were mostly reproduceable in the hold-out set CR-score: 0.62, 95% CI 0.53 – 0.70, p:0.007, CB-score: 0.58, 95% CI 0.49 – 0.67, p=0.07 and CBR-score: 0.61, 95% CI 0.52 – 0.69, p=0.02 and were therefore not plotted in the survival curves (Figure 5).

Figure 5. Concordance indexes for Clinical- Body composition- and Radiomics scores

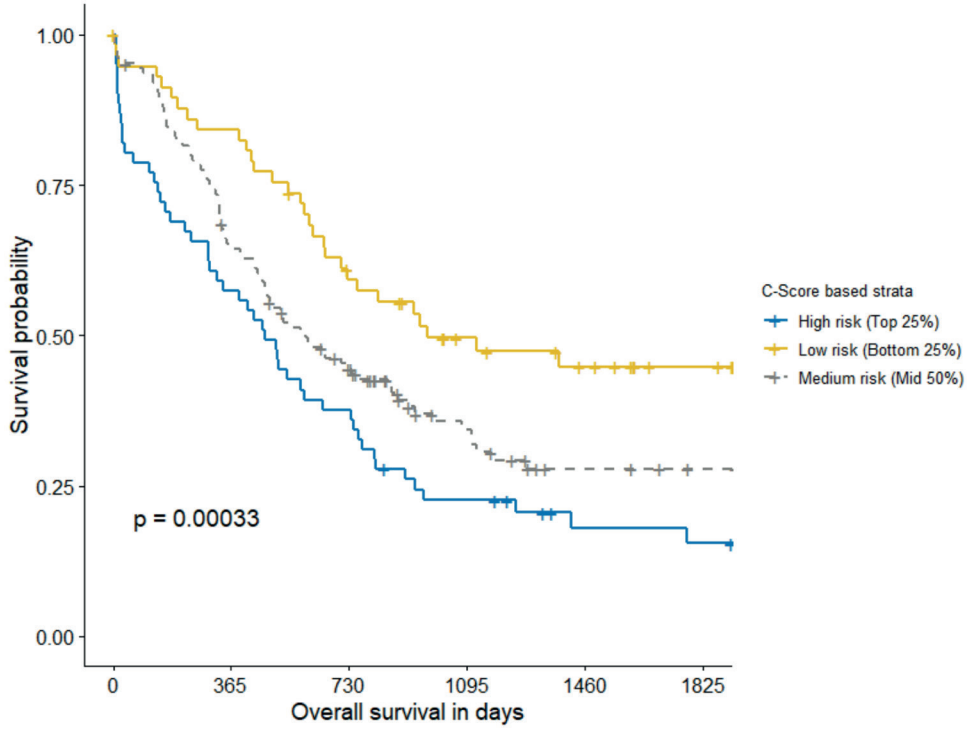


Mean Harrell concordance indexes of Clinical (C-score), body composition (B-score) and Radiomics (R-score) as well as combined clinical + body composition (CB-score), body composition + radiomics (BR-score) and clinical + body composition + radiomics (CBR-score) with corresponding 95% confidence intervals (error bars) and p-values from Log rank testing of both the training set (red) and hold-out-set (blue) are shown.

From the survival curves (Figure 6), it can be seen that C-, B-, and R-scores all produced survival plots which discriminated between patients with an improved and decreased overall survival ($p<0.001$, $p=0.035$, and $p<0.001$, respectively). C- and R-scores showed best discrimination between patients with high, medium, and low risk for worse overall survival. However, the splits of all scores could not be significantly reproduced in the holdout sets.

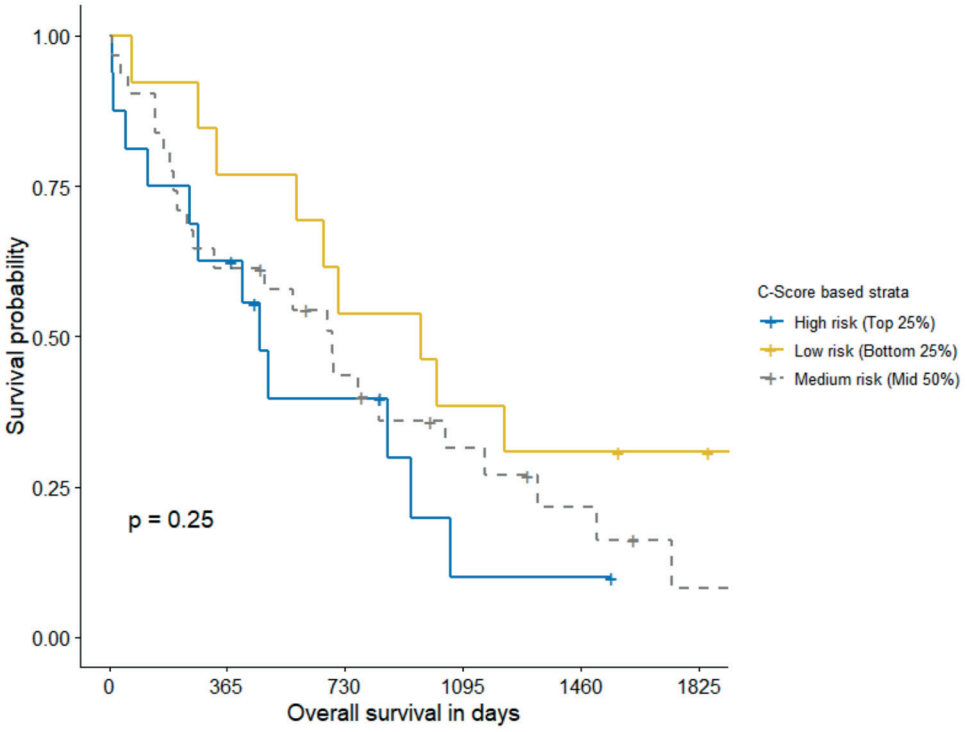
Figure 6.

6a. C-score training set



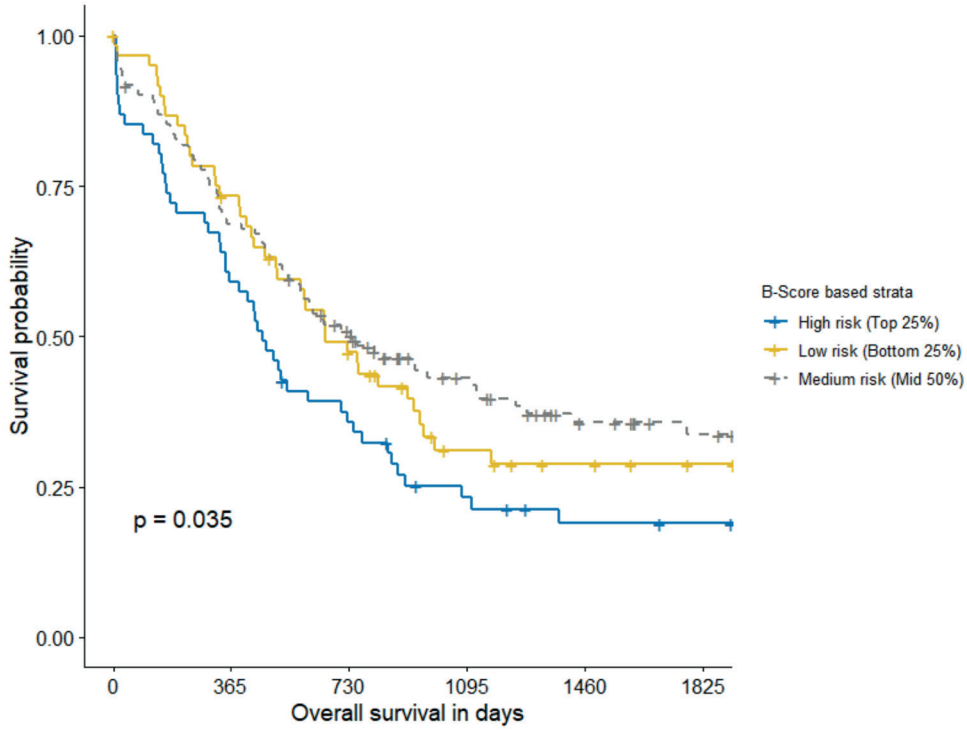
Kaplan-Meier charts with corresponding P-values for C-scores for overall survival following pancreatic head resection for the treatment of PDAC. Blue, yellow and gray lines constitute the highest 25-, lowest 25-, and middle 50 percentages of risk. We computed the Harrell Concordance Index (C-index) for the training set [25]. The fitted regression predictors were used as "scores" for a clinical (C-Score) model. In order to stratify patients into the highest 25% and lowest 25% risk of mortality compared to the middle 50%. A Harrell Concordance Index (c-index) was used as the statistical discrimination metric.

6b. C-score holdout set



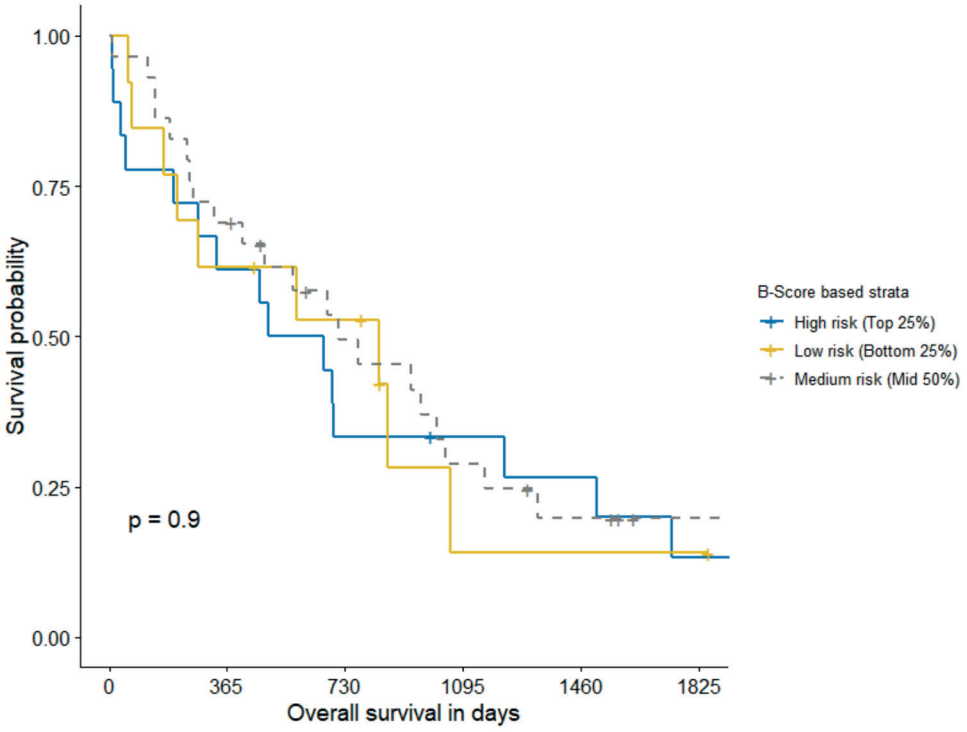
Kaplan-Meier charts with corresponding P-values for C-score for overall survival following pancreatic head resection for the treatment of PDAC. Blue, yellow and gray lines constitute the highest 25-, lowest 25-, and middle 50 percentages of risk. We computed the Harrell Concordance Index (C-index) for the holdout sets [25]. The fitted regression predictors were used as "scores" for a clinical (C-Score) model. In order to stratify patients into the highest 25% and lowest 25% risk of mortality compared to the middle 50%. A Harrell Concordance Index (c-index) was used as the statistical discrimination metric.

6c. B-score training set



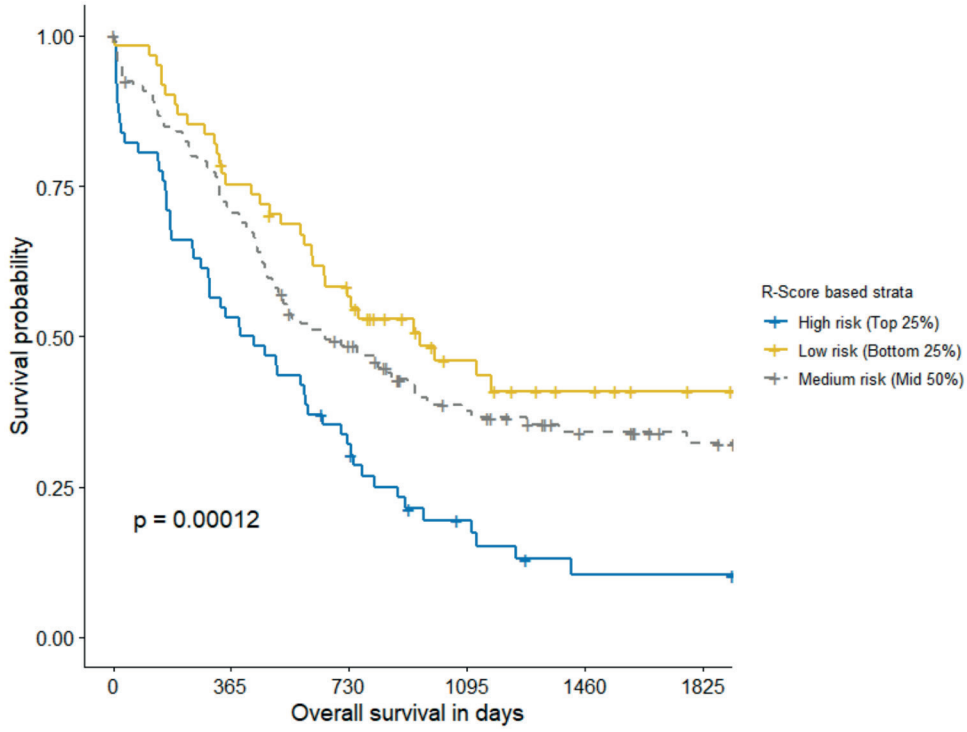
Kaplan-Meier charts with corresponding P-values for B-score for overall survival following pancreatic head resection for the treatment of PDAC. Blue, yellow and gray lines constitute the highest 25-, lowest 25-, and middle 50 percentages of risk. We computed the Harrell Concordance Index (C-index) for the training set [25]. The fitted regression predictors were used as "scores" for a body composition (B-Score) model. In order to stratify patients into the highest 25% and lowest 25% risk of mortality compared to the middle 50%. A Harrell Concordance Index (c-index) was used as the statistical discrimination metric.

6d. B-score hold out set



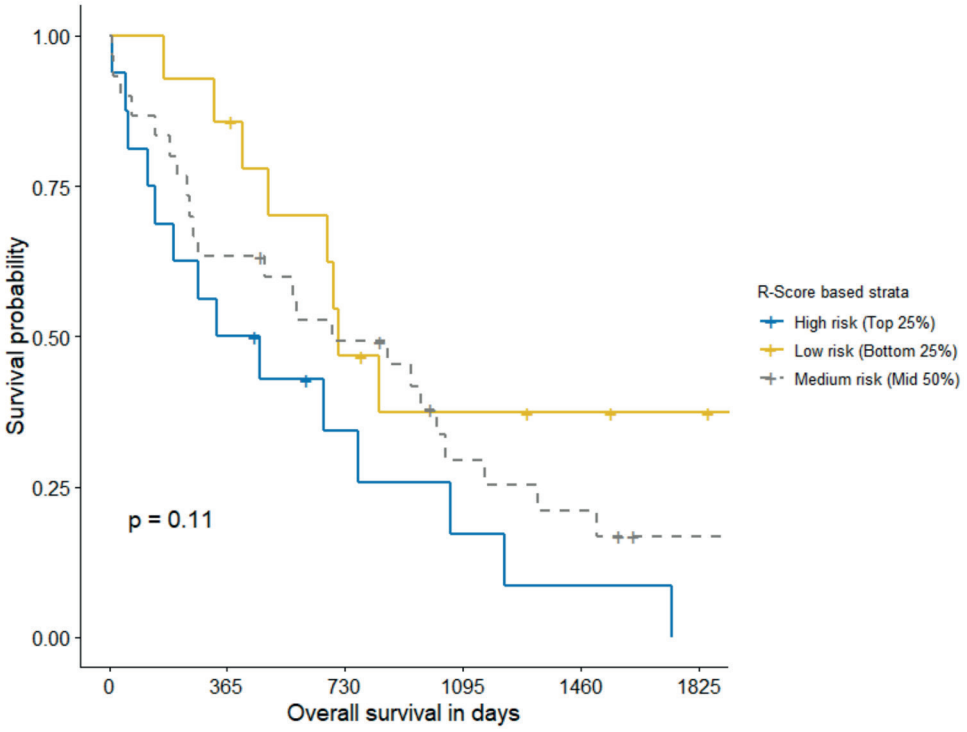
Kaplan-Meier charts with corresponding P-values B-scores for overall survival following pancreatic head resection for the treatment of PDAC. Blue, yellow and gray lines constitute the highest 25-, lowest 25-, and middle 50 percentages of risk. We computed the Harrell Concordance Index (C-index) for the holdout sets [25]. The fitted regression predictors were used as "scores" for a body composition (B-Score) model. In order to stratify patients into the highest 25% and lowest 25% risk of mortality compared to the middle 50%. A Harrell Concordance Index (c-index) was used as the statistical discrimination metric.

6e. R-score training set



Kaplan-Meier charts with corresponding R-scores for overall survival following pancreatic head resection for the treatment of PDAC. Blue, yellow and gray lines constitute the highest 25-, lowest 25- and middle 50 percentages of risk. We computed the Harrell Concordance Index (C-index) for the training set [25]. The fitted regression predictors were used as "scores" for a radiomics (R-Score) model. In order to stratify patients into the highest 25% and lowest 25% risk of mortality compared to the middle 50%. A Harrell Concordance Index (c-index) was used as the statistical discrimination metric.

6f. R-score holdout set



Kaplan-Meier charts with corresponding P-values for R-scores for overall survival following pancreatic head resection for the treatment of PDAC. Blue, yellow and gray lines constitute the highest 25-, lowest 25-, and middle 50 percentages of risk. We computed the Harrell Concordance Index (C-index) for the holdout set [25]. The fitted regression predictors were used as "scores" for a radiomics (R-Score) model. In order to stratify patients into the highest 25% and lowest 25% risk of mortality compared to the middle 50%. A Harrell Concordance Index (c-index) was used as the statistical discrimination metric.

DISCUSSION

In this study, we showed that it is feasible to implement a data-driven radiomics approach to body composition imaging, and that radiomics features can be extracted which perform comparably to conventional body composition variables for the prediction of overall survival of PDAC patients undergoing primary resection. Our data additionally indicate that VAT, SM, and SAT all contain radiomics features with potential predictive information for overall survival.

CT-based analysis of body composition has been validated for the quantification of whole-body muscle mass. This method has become increasingly popular to investigate the association between muscle mass, visceral adipose tissue mass, subcutaneous adipose tissue mass, and patient survival and/or response to treatment [20, 26, 27]. Combined

high visceral adipose tissue mass and low muscle mass have been shown to be associated with increased postoperative morbidity and mortality following oncological pancreatic resection [28]. More recently, it has become evident that SM-RA, a radiological marker of myosteatosis, may be more indicative of wasting and decline of general condition and mortality in certain tumor entities, including pancreatic cancer [7, 29-31]. In the current cohort, however, visceral adiposity was the most important conventional body composition variable associated with overall survival following pancreatic resection. In addition, the radiomics feature signature pool with the highest association with overall survival included features from the visceral and subcutaneous adipose tissue compartments. Although only the highest ranking radiomics features could be selected for cox regression analysis, the runner-up feature pool did contain radiomics features from the skeletal muscle compartment. Thus, although our radiomics model did not contain radiomics features from skeletal muscle VAT, SAT, and SM compartments may all contain radiomics features that hold predictive information for overall survival.

Radiomics imaging analysis originated in tumor imaging studies, where it was shown that large numbers of qualitative and quantitative CT features, which cannot be easily interpreted by the unaided human eye, could supply additional information about tumor heterogeneity, thereby giving a non-invasive estimation of disease severity [14-16, 32]. De Jong et al. recently investigated whether skeletal muscle radiomics features are different in patients who develop muscle loss and those who maintain their muscle function after chemotherapy in a NSCLC cohort [18]. Their study used two timepoints, prior to and following chemotherapy, to investigate changes in muscle mass and did not identify any muscle radiomics features associated with loss of muscle mass during treatment. The above-mentioned study solely extracted radiomics features from skeletal muscle and did not investigate the association between body composition, radiomics, and outcome, or correct for sex specific body composition variation.

Our clinical model (C-score) included age at the time of surgery, ASA-classification, and sex, and our body composition model (B-score) corrected for sex specific differences in body composition by implementing Z-scores. Due to a limited cohort size, splitting the cohort into male and female subgroups for radiomics analysis would have greatly underpowered our radiomics analysis. It was therefore not possible to establish whether sex specific differences in body composition produce different, sex-specific, radiomics signatures. It is however noteworthy, that despite the inability to perform sex-specific radiomics analysis, the radiomics model performed better than the body composition model for prediction of overall survival. This may imply that radiomics signatures are less subject to sex-specific differences than conventional body composition analysis. A possible explanation for this could be that radiomics focusses on textural and signal intensity aspects rather than volume and mass.

We implemented a purely data driven approach to feature selection and modeling. However, it is important to note that predictive models which use a large number of

candidate image features are susceptible to an increased risk of type I errors [32-34]. This particularly holds true for studies with limited cohort sizes. Our cohort size implies a small holdout set. This could explain why all scores that showed a significant split of the Kaplan-Meier curves in the training set could not be reproduced in the holdout set. Although statistical significance was not achieved in the holdout set, we did observe a trend toward significance for the R-score. This could imply that our data may be underpowered to show the discriminatory value of radiomics for overall survival in the holdout set. The limited sample size additionally ruled out including all relevant radiomics features in our time-to-event model. Only one signature pool, containing three features, could be used for the time-to-event model for overall survival. This may have led to an underestimation of the cumulative effect of all relevant features. We were not able to test for repeatability and reproducibility of extracted radiomics feature values due to the fact that we did not have any explicit test-retest sample or multiple expert observers annotating the body compartments independently. However, we are confident that our method of 1000-times replication sampling and nested 10 times repeated 5-fold cross-validation with testing in a holdout set implicitly averaged out most of the instabilities of radiomics features with respect to hospitals, scanners, and imaging protocols. It is also likely that this approach limits the degree of overfitting in the current cohort.

Although body composition (i.e. sarcopenia and visceral adiposity) has been shown to be associated with worse outcome following pancreatic resection, body composition analysis has not found its way into clinical oncological treatment algorithms yet. Our data indicate that it is possible to identify a group of patients at risk for worse outcome based solely on body composition using radiomics. Our cohort, however, did not include patients receiving neoadjuvant- or palliative chemotherapy. Further evaluation of radiomics body composition analysis for the purpose of the identification of body composition-associated risk in chemotherapy treatment groups would provide valuable insights for clinical decision making and for the development of future treatment strategies.

CONCLUSIONS

We found that it is feasible to implement a data-driven radiomics approach to body composition imaging, and we were able to extract radiomics features which held improved predictive value compared to conventional body composition variables for the prediction of overall survival of PDAC patients undergoing primary pancreatic resection. Furthermore, our data shows that VAT, SAT, and SM compartments all contained radiomics features that hold predictive information for overall survival. To gain actionable insight, larger cohort studies are needed to further investigate the added value of radiomics for the prediction of outcome for cancer patients.

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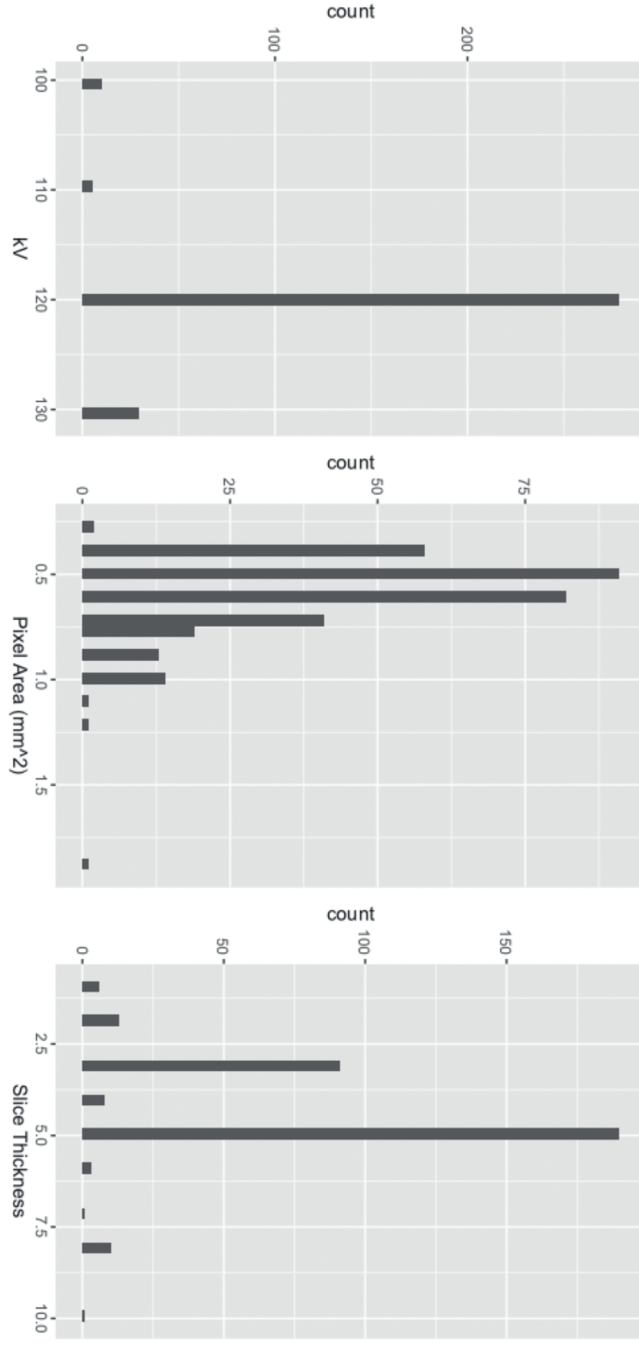
APPENDIX 1, CT-SCANNER SETTINGS.



Figures showing the percentages of different scanner settings Kilovoltage (Kv), Pixel area in mm^2 and slice thickness in mm in our cohort.

CHAPTER

General Discussion

INTRODUCTION

The overall aim of this thesis was to identify cachexia and sarcopenia associated surgical risk. To that end, we investigated the efficacy of nutritional screening tools for the prediction of postoperative morbidity. We went on to use CT based body composition imaging to investigate myosteatosis as a risk predictor of postoperative morbidity following orthotopic liver transplantation. In the context of postoperative morbidity, we investigated thoracic myosteatosis for the identification of patients at risk for postoperative pneumonia following the resection of hepatic colorectal metastasis. Findings from this study led us to develop a prospective observational study entailing the investigation of diaphragm function in sarcopenic and non-sarcopenic patients. Finally, we applied radiomics analysis to investigate whether radiomics-based body composition features can discriminate between patient groups with increased or decreased overall survival following curative resection of the pancreatic head for the treatment of PDAC.

Nutritional risk screening & body composition imaging

In **chapter two,** we investigated the efficacy of nutritional risk screening tools for the prediction of postoperative morbidity, and observed, in accordance with current literature, that malnutrition is associated with increased postoperative morbidity. In addition to morbidity, many studies have shown that malnutrition is associated with reduced survival in a range of oncological diseases [1-9]. Cachexia comprises a spectrum ranging from precachexia, characterised by mild weight loss and anorexia, to cachexia, which is associated with more severe weight loss, sarcopenia and systemic inflammation, and finally refractory cachexia where there is no more response to anti-cancer treatment and life expectancy is less than three months [10]. Prior to reaching its refractory phase, cachexia is not completely irreversible. Often, the reduced food intake can be treated through the active management of nutrition and symptoms negatively impacting intake (i.e. uncontrolled pain or constipation), [11] or with appetite stimulants or artificial nutritional support [12, 13]. Thus, identification and commencement of treatment in the early stages of (pre)cachexia are of paramount importance for the reversal of symptoms. Vital to this early identification of patients at risk is the diligent implementation of nutritional risk screening. However, this constitutes a significant hurdle in the current clinical setting. A cross-sectional nutritional survey of 21,007 patients over 1217 units from 325 hospitals in 25 countries between 2007 and 2008 showed a variable implementation of these screening tools between different units in different countries. A screening routine existed for 93% of units in the United Kingdom while less than 33% of units in Austria, Germany, and the South Eastern European regions reported that they regularly screened patients for malnutrition on admission [14]. Although these data are over a decade old and may therefore not reflect the current state of screening, it is plausible to consider that regional differences in diligent implementation of nutritional risk screening exist and should be

addressed in order to ensure an optimal treatment potential. It also underscores the need for a more up-to-date European cross-sectional nutritional survey.

We compared the use of CT-based body composition analysis with nutritional risk screening tools for the identification of patients at risk for increased postoperative morbidity in **chapter three**. Our data indicated that nutritional risk screening tools performed better than CT-based body composition analysis for the prediction of postoperative morbidity following oncological colorectal resection. However, the data failed to show significant differences in multivariable analysis. The limited cohort size of this study constitutes an important limitation and makes multivariable analysis underpowered. That being said, studies prospectively comparing nutritional risk screening and body composition imaging for the prediction of postoperative risk are not abundant. Reisinger et al. found that there was no significant association between sarcopenia alone and sepsis after CRC surgery, but postulated that a combined risk stratification using sarcopenia, functional compromise (measured by Groningen Frailty Index), and nutritional risk (measured by Short Nutritional Assessment Questionnaire) could successfully predict sepsis [15]. Huang et al. did not find nutritional risk screening (NRS-2002) to be predictive of postoperative morbidity in a colorectal cancer cohort. They argued that sarcopenia should be defined as reduced muscle mass and reduced muscle strength, and/or physical performance. Their work also showed that sarcopenia measured as reduced muscle mass and reduced physical performance (measured by gait speed and handgrip strength) were both independent predictors of post-operative complications after CRC surgery [16].

Simonsen et al. performed a meta-analysis including 7176 patients who underwent gastrointestinal oncological surgery and showed that sarcopenia was an independent risk factor for increased postoperative complications [17]. Subgroup analysis showed that of the sarcopenia assessment methods used amongst different studies (SMI, total psoas volume (TPV), total psoas index (TPI), and bioelectrical impedance analysis (BIA)), all assessment methods with the exception of TPV remained significant risk factors for postoperative complications. Further subgroup analysis on the application of sarcopenia cut-off values was performed by comparing studies using predefined cut-off values or subgroup specific or optimum stratification cut-offs. In studies using study-data to define sarcopenia cut-off values, sarcopenia remained significantly associated with an increased risk of postoperative complications, whilst in studies using predefined sarcopenia cut-off values, sarcopenia was not a significant risk factor. Simonsen went on to investigate whether different sarcopenia criteria used amongst studies influenced the estimation of postoperative risk. Their subgroup analysis showed that sarcopenia remained a significant risk factor of postoperative complications independent of the sarcopenia criteria being used. However, interestingly, they did find significant differences between studies using muscle mass and EWGSOP criteria for the detection of sarcopenia, with studies using EWGSOP criteria showing a significantly higher increase in risk ratio. This illustrates the

importance of assessing muscle strength and physical performance for the detection of sarcopenia.

In 2019, the EWGSOP published their revised European consensus on definition and diagnosis of sarcopenia [18]. The new definition focuses on low muscle strength as a key characteristic of sarcopenia. It uses detection of low muscle quantity and quality to confirm the sarcopenia diagnosis, and identifies poor physical performance as indicative of severe sarcopenia. The EWGSOP puts forward a number of clinical tools for the evaluation of muscle strength (handgrip strength and chair rise test), which have been shown to accurately quantify whole body muscle strength [19]. Physical performance has been defined as an objectively measured whole-body function related to locomotion. This is a multidimensional concept that not only involves muscles but also central and peripheral nervous function, including balance [20]. The EWGSOP recommends a number of tests for the evaluation of physical performance (chair rise test, SPPB, and gait speed).

Chapter two and **three** encompass the need for early detection of malnutrition, sarcopenia and myosteatosis by way of nutritional risk screening and body composition imaging. Our work, in line with current literature, underlines the importance of implementation of screening in daily clinical practice. Future work regarding the role of sarcopenia and its effect on the response to treatment should not focus solely on imaging for the evaluation of muscle mass and muscle quality, but rather endeavour to incorporate functional muscle tests into the clinical routine.

Sarcopenia and liver disease

Sarcopenia is a common complication of hepatic cirrhosis and is observed in up to 60% of patients with end stage liver disease (ESLD). It portends a poor prognosis in patients with ESLD. Liver cirrhosis is characterized by accelerated starvation with an impaired adaptive response to fasting, due to impaired hepatic function. In patients with cirrhosis, fatty acid oxidation and muscle and hepatic glycogen reduction occurs within 10 hours of fasting in an equivalent manner to what would be observed in healthy subjects after 3 days of starvation [21].

A broad literature has described a link between sarcopenia and myosteatosis, and outcome in end stage liver disease [22-26]. In addition, clinical studies have shown sarcopenia to be a risk factor for outcome following liver transplantation [27-30]. Krel et al. previously showed that sarcopenia measured by total psoas area (TPA) was associated with serious infections following liver transplantation [28]. Englesbe et al. showed that TPA was associated with mortality following liver transplantation [30]. Esser et al. compared several sarcopenia classifiers including TPA, Psoas Density (PD), and skeletal muscle density (i.e. SM-RA) and concluded that PD and SM-RA were associated with increased complication

severity [31]. The above studies demonstrate an association between sarcopenia and outcome following liver transplantation but have some shortcomings regarding body composition imaging methodology. The use of psoas muscle or any other sentinel muscle for the diagnosis of sarcopenia is debated, and, in contrast to L3 measurements, not a validated tool for the measurement of total body muscle mass [32, 33].

Risk scores for end stage liver disease and the role of sarcopenia & myosteatosis

The most important risk predictor in liver transplantation is the Model for End stage Liver Disease (MELD)-score in Europe and north America. The MELD-score is used for patient prioritization and organ allocation in end-stage liver disease and is validated to predict 3-month waiting list mortality. The score is calculated using serum levels of bilirubin, creatinine and the international normalized ratio (INR) [34]. Despite its strong predictive value, the MELD score underestimates disease severity in about 15–20% of patients with cirrhosis, resulting in an inaccurate prediction of survival [35]. A frequently reported drawback of the MELD score is the lack of an objective parameter reflecting patients' physical and nutritional status. Consequently, patients with a low MELD score, but with malnutrition or sarcopenia, may be under prioritised in the current system [36]. Recently, adaptation of the currently used MELD-score to include sarcopenia to more accurately predict waiting list mortality has been proposed.

Several studies have demonstrated that sarcopenia is associated with increased waiting list mortality [37, 38]. Subsequently, Montano-Loza et al. found a significantly shorter waiting list survival in patients with sarcopenia, and therefore included sarcopenia in the MELD-score (MELD-Sarcopenia score). This score showed a higher predictive accuracy for waiting list mortality than the MELD-score alone in their north American cohort [39]. More recently, Van Vught et al. investigated the added value of a Sarcopenia-MELD-score in a Dutch National cohort. Their rigorous analysis constituted an external validation of Montano-Loza et al.'s sarcopenia-MELD score and contained a competing risk analysis in a homogenous waiting list cohort. Their data indicated that sarcopenia was associated with waiting list mortality in liver transplant candidates with cirrhosis, particularly in patients with lower MELD-scores. The MELD-Sarcopenia score was successfully validated in their cohort. However, the group concluded that incorporating sarcopenia in the MELD score had limited added value in predicting waiting list mortality [40].

As noted above, the MELD-score calculates preoperative waiting list mortality based on recipient factors. In 2011, Dutkowski et al. proposed an alternative to the MELD-score which includes donor and recipient factors, the Balance of Risk (BAR)-score [41]. Their group suggested that the BAR-score outperformed the MELD-score for the prediction of post-transplant survival [41]. However, the same drawbacks reported for the MELD-score, namely the lack of an objective parameter reflecting patient physical status, also holds true for the BAR-score.

The above-mentioned works focussed on sarcopenia as a measure of muscle wasting but did not incorporate myosteatosis in their body composition analysis. Although several studies have shown that myosteatosis negatively affects outcome in cirrhotic patients, not much data is available about its impact on outcome in liver transplantation [23]. In **chapter four,** we investigated whether sarcopenia and myosteatosis were associated with postoperative morbidity following deceased donor liver transplantation. In this study, myosteatosis was significantly associated with poor perioperative outcome including morbidity, mortality, EAD (Early Allograft Dysfunction), ICU- and hospital stay. In addition, our data showed that with the introduction of myosteatosis to the existing BAR-score (BAR-myosteatosis-score), better predictability of morbidity and mortality was achieved. This work adds to the current literature by demonstrating the clinical importance of myosteatosis in a liver transplant cohort, and indicates that myosteatosis, which is thought to reflect muscle function, might be of greater clinical importance than muscle mass (sarcopenia) in a liver transplant cohort.

Chapter four encompasses the importance of sarcopenia and myosteatosis in a liver transplant population. Although our data indicates that myosteatosis is of greater importance than sarcopenia in this setting, other studies in similar cohorts failed to investigate or show the importance of myosteatosis. To that end, future studies should focus on this aspect of wasting disorders in this patient population. As the MELD-score is still the most common tool used for organ allocation, efforts should be made to investigate the added value of myosteatosis in the form of a modified MELD-myosteatosis score to potentially include patient physical condition and wasting disorders in organ allocation algorithms. As the clinical importance of wasting disorders and physical condition in liver transplant patients is becoming more evident, the use of the preoperative period could potentially be utilised to improve patient fitness. This is known as prehabilitation. Recently, van Berkel et al. investigated the effects of community based-exercise in the form of prehabilitation on high-risk colorectal patients in a randomized controlled trial [42]. The group concluded that exercise prehabilitation reduced postoperative complications in high-risk patients scheduled for elective colorectal colon resection. Van Wijk et al. prospectively evaluated the screening and assessment of modifiable risk factors amendable for pre- habilitation interventions. Their results indicated that it is feasible to implement a structured screening and assessment for six modifiable risk factors by reengineering the preoperative care pathway. Two thirds of patients who were eventually scheduled for HPB cancer surgery needed preoperative optimisation of one or more risk factors. However, their group reported a compliance of 53%, indicating that there is much room for improvement in this difficult patient population. We hypothesize that such compliance challenges may also play an important role in a transplant cohort and propose that future works should include these cohorts. With the announcement of a substantial Grant from the Dutch Kidney Foundation, the University Medical Center Groningen has

recently begun the design of a prehabilitation study in a kidney transplant cohort which may provide further insight into patient preconditioning in a transplant cohort.

Lifestyle interventions & treatment

As in oncology, implementation of tools enabling early detection of sarcopenia and myosteatosis, as described in the previous section, are of paramount importance to identify patients with increased perioperative risk. The subsequent interventions for patients with primary liver disease may however differ from those in oncological patients. For instance, weight loss achieved by diet and exercise is the mainstay of NASH management. The amount of weight loss is directly proportional to the degree of improvement in liver histology [43]. The recommendation for weight loss in patients with sarcopenia, however, should be carefully balanced with nutritional status. Those with sarcopenic obesity may benefit from caloric restrictions, whereas patients with normal or low BMI may be harmed by this recommendation. Management of sarcopenia is therefore complex in this population. Patients with cirrhosis have an accelerated state of catabolism with fasting and should be encouraged to snack frequently [44]. Supplementation with protein-based calories has been shown to improve muscle mass and reduce protein catabolism [45, 46].

Adipose tissue & visceral adiposity

Loss of adipose tissue in cancer cachexia results primarily from increased lipolysis and can happen earlier than loss of skeletal muscle tissue [47, 48]. Low subcutaneous- and visceral adipose tissue mass have both been shown to be associated with worse survival in cancer patients [49]. In addition, in the presence of sarcopenia, increased subcutaneous adipose tissue has been shown to be associated with improved survival [50]. In contradiction, some studies involving pancreatic cancer and hepatocellular carcinoma patients have shown that increased visceral adipose tissue (visceral adiposity) is associated with increased postoperative morbidity and reduced survival [51-53].

In **chapter seven,** we implemented a radiomics approach to body composition imaging and compared the performance of radiomics features to known body composition imaging features (VATI, SATI, SMI and SM-RA) for the prediction of survival. In this study, we observed that increased visceral adiposity (VATI) emerged as the sole body composition variable associated with reduced overall survival, illustrating that different body composition profiles constitute diversity of risk amongst different cancer types. In the paragraph entitled *radiomics*, which can be found below, we will elaborate further on the findings of the above-mentioned research.

Pulmonary morbidity, diaphragm function

Pulmonary morbidity, especially postoperative pneumonia, is of particular importance in surgery and has been shown to prolong hospital admission and increase in-hospital mortality following a range of surgical interventions, most notably after major abdominal

and upper gastro-intestinal surgery [54-58]. More specifically, patients undergoing partial hepatectomy frequently develop reactive pleural effusion, which increases the risk of post-operative pneumonia to incidences above 10% [54, 56-61]. In **chapter five,** we investigated whether reduced SM-RA of the thoracic- (T4 SM-RA) and abdominal compartments (L3 SM-RA) is associated with increased postoperative pneumonia. We found that low L3 SM-RA and T4 SM-RA showed similar odds-ratios and were both associated with postoperative pneumonia. It can therefore be argued that T4 SM-RA reflecting thoracic myosteatosis may be a valuable body composition parameter, in particular when no recent abdominal scan is available.

The abovementioned study investigated myosteatosis at the fourth thoracic vertebrae for the prediction of pneumonia. This study brought to light a shortcoming of CT body composition analysis in the context of the prediction of pulmonary morbidity, which is that in our experience, it is not practicable to use CT-imaging for the purpose of investigating diaphragm morphology or function. This knowledge led to the design of a prospective study which is described in **chapter six.** This currently ongoing study uses trans-costal ultrasound for the perioperative investigation of diaphragm function**.** Interestingly, very little is known about the function of the diaphragm in the context of sarcopenia and wasting disorders or how its function is influenced by abdominal surgery. Some preclinical animal studies have shown that sarcopenia is associated with atrophy of diaphragmatic muscle fibers, and that ageing is related to a decline in diaphragmatic function [62-65]. Clinical studies in the context of prolonged mechanical ventilation of acutely ill patients have focused on diaphragm function using ultrasound technology and have shown that prolonged ventilation can lead to diaphragm atrophy which is associated with worse clinical outcome [66-68]. The primary goal of the study described in **chapter six** is to evaluate whether sarcopenic patients have a reduced diaphragm function prior to major liver resection compared to non-sarcopenic patients, and to evaluate whether sarcopenic patients show a greater reduction in respiratory muscle function following major liver resection when compared to non-sarcopenic patients. The results of this trial will contribute to the understanding of the role of the diaphragm in pulmonary morbidity following major liver resection and will contribute to identifying patients who are at risk for pulmonary complications following major liver resection.

Radiomics

The field of radiomics was developed assuming that advanced image analysis using medical imaging modalities could capture additional information not currently used in diagnostics [69]. Radiomics features have been shown to provide prognostic value in predicting clinical outcomes of several tumor entities, including head and neck cancer and lung tumors [70-72]. It has been demonstrated that major differences in protein expression patterns within a tumour can be correlated to radiographic findings (or radiophenotypes) based on CT data [73].

De Jong et al. recently investigated whether skeletal muscle radiomics features are different in patients who develop muscle loss versus those who maintain their muscle function after chemotherapy in a Non-Small Cell Lung Carcinoma cohort [74]. Their study used two timepoints, prior to and following chemotherapy, to investigate changes in muscle mass and did not observe any muscle radiomics features associated with loss of muscle mass during treatment. The abovementioned study solely extracted radiomics features from skeletal muscle and did not investigate the association between body composition, radiomics, and outcome. In **chapter seven,** we investigated whether radiomics-based body composition features can discriminate between patient groups with improved or reduced overall survival following curative resection of the pancreatic head for the treatment of PDAC (Pancreatic Ductal Adenocarcinoma). Our data indicated that it is feasible to implement a data-driven radiomics approach to body composition imaging, and we were able to extract radiomics features which seem to hold comparable or slightly improved predictive value compared to conventional body composition variables for the prediction of overall survival of PDAC patients undergoing primary resection. Furthermore, our data shows that VAT (Visceral Adipose Tissue), SM (Skeletal Muscle), and SAT (Subcutaneous Adipose Tissue) compartments all contained radiomics features that appear to hold predictive information for overall survival. It must be noted that verification in a limited hold-out set did not unequivocally reproduce the above-mentioned outcome, likely due to a limited cohort size.

Large cohort studies will be needed to ascertain the added value of radiomics in the clinical setting, and, eventually, raise the question of its possible role in daily clinical practice. Several challenges regarding application of such technology can be expected. The foremost, which already challenges the application of conventional body composition imaging in daily practice, is the practical issue of image segmentation. All images used for body composition imaging are either segmented by hand or segmented using semi-automated segmentation software which needs to be corrected by hand. Such image analysis is time consuming and has therefore not found its way into clinical routines. If body composition imaging of any kind is to play a role in clinical routines, fully autonomous image segmentation software is needed. If such image autonomous image segmentation could be performed real-time, clinical applicability, for instance in the setting of multidisciplinary oncological conferences could be practicable, thus playing a role in oncological treatment planning. A further challenge specifically associated with radiomics-based/assisted body composition imaging pertains to the vast amount if radiomics features which are extracted. Conventional body composition imaging yields a handful of relatable features, i.e. volume, mass and density. Radiomics analysis, on the other hand, produces a set of arbitrary features which can be associated with a range of computed image characteristics based on texture, signal-intensity and shape. Such image features are largely unrelatable to a medical clinician, thus creating a disparity between image-data and clinical interpretability. Another issue which further complicates the

applicability of radiomics analysis in general, which may also hold true for radiomics body composition imaging, is that each tumor entity produces a unique set of radiomics features associated with outcome. Whether this is the case for radiomics-based body composition analysis, or whether patients eliciting wasting disorders show a reproduceable set of radiomics features amongst different cohorts remains to be seen.

Perhaps the most significant challenge for the clinical implementation of radiomics and conventional body composition imaging is that imaging outcome needs to produce actionable insight. Future works should therefore focus not only on risk stratification based on body composition characteristics, but rather focus on therapeutic consequence, such as adaptation of chemotherapeutic dosing based on body composition or selecting at risk patients for early optimisation of the preoperative planning to allow for a patient tailored prehabilitation.

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CHAPTER

Impact

The previous chapter focused on the main findings of this thesis whilst placing them in the context of current literature. This concluding paragraph will give a reflection on the practical, societal, and scientific impact of the results described in this thesis.

Early identification

Cachexia and sarcopenia profoundly impact short- and long-term outcome in oncological surgery [1-6]. Our work underlined the importance of nutritional risk screening in a surgical cohort, thus adding to the basis of evidence that early recognition with the aid of nutritional risk screening, as well as early clinical identification of sarcopenia using physical activity testing is of clinical importance.

Pulmonary morbidity & myosteatosis

Pulmonary morbidity, especially postoperative pneumonia, is of particular importance in surgery and has been shown to prolong hospital admission and increase in-hospital mortality following a range of surgical interventions, most notably after major abdominal and upper gastro-intestinal surgery [9-13]. Our research showed that myosteatosis of the thoracic and abdominal compartments is associated with increased postoperative pneumonia. Our work adds to current evidence regarding the importance of myosteatosis by indicating that myosteatosis can be measured in other body compartments than abdominal at the third lumbar vertebrae, and that this holds clinical value. This study brought to light a shortcoming of CT body composition analysis in the context of the prediction of pulmonary morbidity, which is that in our experience, it is not practicable to use CT-imaging for the purpose of investigating diaphragm morphology or function. This knowledge led to the design of a prospective study. This currently ongoing study hypothesizes that reduced muscle function in the context of wasting disorders as myosteatosis and sarcopenia effect pulmonary function as a consequence of reduced diaphragm function. The study uses trans-costal ultrasound for the perioperative investigation of diaphragm function**.** The results of this trial will contribute to the understanding of the role of the diaphragm in pulmonary morbidity following major liver resection, and will contribute to identifying patients who are at risk for reduced pulmonary function following major liver resection.

Myosteatosis and liver disease

Numerous studies have described the pathophysiological and clinical relationships between liver dysfunction and sarcopenia and myosteatosis [14-18]. Our work added to this by showing that myosteatosis negatively impacts patients undergoing liver transplantation. More specifically, that myosteatosis is associated with increased postoperative complications, ICU-stay, admission-time, mortality, and healthcare costs following liver transplantation. Future studies should focus on this aspect of wasting disorders in this patient population. As the MELD-score is still the most common tool used

for organ allocation, efforts should be made to investigate the added value of myosteatosis in the form of a modified MELD-myosteatosis score to potentially include patient physical condition and wasting disorders in organ allocation algorithms.

Radiomics

The field of radiomics uses advanced image analysis to capture additional information not currently used in conventional medical imaging. We investigated whether radiomics-based body composition features can discriminate between patient groups with improved or reduced overall survival following curative resection of the pancreatic head for the treatment of PDAC (Pancreatic Ductal Adenocarcinoma). Our work indicated that it is feasible to implement a radiomics approach to body composition imaging, and we were able to extract radiomics features which seem to hold similar predictive value compared to conventional body composition variables for the prediction of overall survival of PDAC patients undergoing primary resection. These findings constitute a novel approach to body composition imaging, and thus will contribute to the discussion whether radiomics analysis holds merit, and whether it may have a place in academic and or daily clinical practice.

Impact on the healthcare system & future perspectives

Cachexia, sarcopenia, and myosteatosis reflect the frailty of our patients. It is well established that the frail patient greatly impacts our health care system in terms of resources and costs. Yet, not all western European healthcare systems have been able to diligently implement basic tools such as nutritional risk screening and sarcopenia screening as a part of standardized practical treatment protocols. This has been shown in a cross-sectional nutritional survey of 21,007 patients over 1217 units from 325 hospitals in 25 countries. A screening routine existed for 93% of units in the United Kingdom while less than 33% of units in Austria, Germany and the South Eastern region reported that they regularly screened patients for malnutrition on admission [19]. Although these data are over a decade old and may therefore not reflect the current state of screening, it is plausible to consider that regional differences in diligent implementation of nutritional risk screening exist and this should be addressed in order to ensure an optimal treatment potential. It also underscores the need for a more up-to-date European cross-sectional nutritional survey.

The work put forward in this thesis does not directly impact patients or the healthcare system as such. However, it does illustrate the importance of a holistic approach to patient care. It emphasizes the need to identify our most fragile patients, who carry the greatest amount of risk. Our currently ongoing study using trans-costal ultrasound for the perioperative investigation of diaphragm function may enable us to identify patients who are at risk for reduced diaphragm function preoperatively, possibly allowing early

preconditioning of patients and therewith an improvement in postoperative pulmonary function. Preconditioning of patients may also be the next step for sarcopenic and myosteatotic patients undergoing liver transplantation. Evaluation of such treatment regiments should be the next step in improving outcome for our most fragile patients.

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Addendum

ENGLISH SUMMARY

Introduction

Cachexia is defined as a multifactorial syndrome characterised by involuntary progressive weight loss as a result of a reduction of skeletal muscle mass (sarcopenia) with or without the depletion of adipose tissue. It is characterized by a spectrum of symptoms ranging from mild weight loss and anorexia to severe weight loss, sarcopenia, and systemic inflammation. The incidence among cancer patients varies according to cancer type, with indices of up to 80% for gastric and pancreatic cancer patients, 50% for patients with lung, colon, or prostate cancer, and around 40% for patients with breast cancer or leukemia.

The pathogenesis of cancer cachexia is multifactorial and characterized by a negative protein and energy balance. Skeletal muscle catabolism leading to reduced muscle mass is thought to be induced by both tumor- and host-derived factors. Fat can accumulate in skeletal muscles in the form of intramyocellular lipid droplets and intermuscular adipocytes, a phenomenon known as myosteatosis. Cachexia, sarcopenia and myosteatosis have been shown to have significant negative impact on short- and long-term outcome and postoperative morbidity following a range of oncological treatments. Where sarcopenia is an indicator of muscle mass, myosteatosis seems to be more associated with impaired muscle function. These conditions are also described as wasting disorders.

Several tools, ranging from easy to-use questionnaires to more complex computed tomography (CT) image analysis have been developed to identify patients who are at risk for, or display symptoms associated with these conditions. For the purpose of this thesis, we will elaborate on the different tools which are commonly used to identify cachexia and sarcopenia associated surgical risk in both the clinical and academic setting. We will also investigate some experimental methods not previously described.

Nutritional risk screening

In oncology and oncological surgery, nutritional risk screening is of vital importance for the identification of cachectic patients, and commencement of appropriate, early nutritional intervention. In chapter two, we performed a prospective observational study to investigate the association between nutritional risk and postoperative morbidity. The results of this study showed that nutritional risk screening, in the form of the MUST (Malnutrition Universal Screening Tool) was an independent predictor for post-operative complications and increased complication severity as measured by the Clavien-Dindo score in patients undergoing elective colorectal surgery, (OR 3.82; 95% CI 1.48-9.88) and (OR 2.78; 95% CI 1.13-6.79) respectively. This work underlined the relevance of adequate nutritional risk screening in elective colorectal surgery.

Computed Tomography (CT) imaging is often used in the academic setting for the detection of sarcopenia. This method uses CT images at the level of the third lumbar vertebra (L3) to determine cross-sectional area of skeletal muscle (SM), visceral adipose

(VAT) tissue, and subcutaneous adipose tissue (SAT), providing good estimates of total body SM-, VAT-, and SAT-mass. Besides the estimation of mass for the detection of sarcopenia, CT image analysis can also be used for the estimation of muscle density, also known as skeletal muscle radiation attenuation (SM-RA). SM-RA is considered to be a radiological marker indicative of myosteatosis.

In *chapter three* we aimed to assess whether CT-measured sarcopenia and/or myosteatosis have additional value besides the MUST for the prediction of postoperative complications after oncological colorectal resection. Results from this prospective observational study showed a high prevalence of sarcopenia in patients undergoing oncological colorectal resection (52%) but failed to show a significant association with post-operative complications. The MUST was univariately associated with postoperative complications ($p<0.05$), myosteatosis approached significant association ($p=0.05$), and sarcopenia did not show a significant association in patients undergoing elective oncological colorectal resection ($p=0.59$). When including both MUST and myosteatosis in multivariable analyses, both failed to show a significant association with postoperative complications ($p=0.12$, OR 4.1, 95%CI 0.7-24.7 and $p=0.18$, OR 0.5, 95%CI 0.1-1.4, respectively). The limited cohort size of this study constitutes an important limitation and made multivariable analysis underpowered.

Chapter two and - *three* highlight the need for early detection of malnutrition, sarcopenia and myosteatosis by way of nutritional risk screening and body composition imaging. Our work, in line with current literature, underlines the importance of the implementation of screening in daily clinical practice. Although our study failed to demonstrate significant associations between sarcopenia, myosteatosis and worse clinical outcome, several studies have since underlined a strong association, thus emphasizing the clinical importance of wasting disorders.

Sarcopenia and liver disease

In the previous chapter, we investigated the importance of nutritional risk screening and screening for sarcopenia and myosteatosis in an onco-surgical cohort. Besides playing an important role in the above-mentioned setting, sarcopenia is a common complication of hepatic cirrhosis and is observed in up to 60% of patients with end stage liver disease (ESLD). It is characterized by accelerated starvation with an impaired adaptive response to fasting, due to impaired hepatic function. The only effective treatment for patients suffering from ESLD is liver transplantation. However, waiting times for a transplant organ can be extensive, with one in six patients dying whilst waiting for a new organ. Several studies have demonstrated that sarcopenia is associated with increased waiting time mortality, as well as post-transplant mortality. However, current literature focussed on sarcopenia as a measure of muscle loss, but did not incorporate myosteatosis in body composition analyses. Several risk predictors, such as the Model for End stage Liver Disease (MELD)-score and the Balance of Risk (BAR)-score are used to calculate preoperative

waiting list mortality. However, these risk scores lack an objective parameter reflecting patient physical status.

In *chapter four,* we performed a retrospective observational study investigating the predictive value of myosteatosis for postoperative morbidity following deceased donor liver transplantation. In this study, myosteatosis was significantly associated with major morbidity (Clavien Dindo≥3b) (OR 2.772, 95%CI 1.516-5.066, p=0.001) in multivariable analysis. In addition, our data showed that with the introduction of myosteatosis to the existing BAR-score (BAR-myosteatosis-score), better predictability of 90-day morbidity and -mortality was achieved (AUROC increase from 0.677 and 0.821 (original BAR score) to 0.710 and 0.853 (BAR-myosteatosis-score). This work adds to the current literature by demonstrating the clinical importance of myosteatosis and indicates that in a liver transplant cohort, myosteatosis indicative of impaired muscle function might be of greater clinical importance than sarcopenia which is indicative of muscle mass.

Sarcopenia, myosteatosis and pneumonia

The previous chapter brought forward the importance of myosteatosis (impaired muscle function) over sarcopenia (muscle mass). Postoperative pneumonia is an important cause of postoperative morbidity and mortality. In *chapter five***,** we performed a prospective observational study investigating the association between myosteatosis at the third lumbar (L3) and fourth thoracic vertebrae (T4) with the incidence of postoperative pneumonia. We found that L3 - and T4 myosteatosis both were equally associated with postoperative pneumonia in a multivariable model (OR 3.65, 95% CI 1.41-9.49, p<0.01 and OR 3.22, 95% CI 1.20-8.61, p=0.02, respectively).

The process of investigating pulmonary morbidity led us to the analysis of the diaphragm. Interestingly, very little is known about the function of the diaphragm in the context of sarcopenia and wasting disorders, or how its function is influenced by abdominal surgery. We therefore designed a prospective observational study, described in *chapter six*, which investigates the function of the diaphragm during the perioperative period using trans-costal ultrasound. In this currently still active study, we are evaluating differences in diaphragm function and recovery between sarcopenic and non-sarcopenic patients undergoing major liver resection. The results of this prospective observational study will contribute to the understanding of the role of the diaphragm in pulmonary morbidity following major liver resection and will contribute to identifying patients who are at risk for pulmonary complications.

Radiomics & body composition imaging

In previous chapters, CT-images were used to extract information about muscle area, muscle density as well as intra-abdominal fat area, to produce a quantification of whole-body muscle mass (sarcopenia) and muscle density (myosteatosis). This method has been

widely described as the gold standard for sarcopenia and myosteatosis analysis using CT-images.

Radiomics analysis extracts large amounts of image data from medical images. Where conventional image analysis focusses on a handful of image properties which are interpreted by medical professionals, radiomics analysis extracts hundreds and sometimes thousands of image properties (radiomics features). This yields large amounts of quantitative image data which in turn is interpreted using complex data-analysis. Associations between image data which cannot be seen with the naked eye, and clinical outcome are subsequently analyzed. Radiomics analysis finds its origins in tumor imaging, where aspects of tumor growth and aggressiveness have been successfully captured using radiomics analysis, bringing forth additional information over conventional interpretation by medical professionals.

In *chapter seven,* we explored the implementation of novel radiomics approaches to the field of body composition imaging. We retrospectively investigated whether the quantification of large amounts of body composition imaging data could provide added value for the identification of patients at risk for reduced survival following oncological pancreatic resection. Our data indicated that it is feasible to implement a data-driven radiomics approach to body composition imaging. We created three models: a Radiomics model (R-score) containing only radiomics features, a clinical model (C-score) containing only clinical data, and a body composition model (B-score) containing only conventional body composition variables. Models were then compared in a training and validation cohort. The mean Harrell concordance-indices for overall survival time models were highest for the radiomics model or R-score (0.61, 95% CI 0.56 – 0.65, $p<0.001$), followed by the clinical model containing only clinical data or C-score (0.59, 95% CI 0.55 - 0.63, $p<0.001$) and finally the conventional body composition score containing muscle mass, muscle density and fat mass named B-score (0.55, 95% CI 0.50 – 0.60, $p=0.03$). These data indicate that radiomics features seem to hold comparable or slightly improved predictive value compared to conventional body composition variables. In addition, our data shows that visceral adipose tissue, skeletal muscle, and subcutaneous adipose tissue compartments all contained radiomics features, or image data, that appear to hold predictive information for overall survival. However, these results did not all remain significant in the independent validation cohort.

CONCLUSION

The work put forward in this thesis illustrates the importance of a holistic approach to patient care. It emphasizes the need to identify our most fragile patients, who carry the greatest amount of risk. Our currently ongoing study using trans-costal ultrasound for the perioperative investigation of diaphragm function may enable us to identify patients who are at risk for reduced diaphragm function preoperatively, possibly allowing

early preconditioning of patients and therewith an improvement in postoperative pulmonary function. Preconditioning of patients may also be beneficial for sarcopenic and myosteatotic patients undergoing liver transplantation. Evaluation of such treatment regiments should be the next step in improving outcome for our most fragile patients. Our work into radiomics analysis constitute a novel approach to body composition imaging, and thus will contribute to the discussion whether radiomics analysis holds merit, and whether it may have a place in academic and/or daily clinical practice.

NEDERLANDSE SAMENVATTING

Introductie

Cachexie wordt gedefinieerd als een multifactorieel syndroom dat wordt gekenmerkt door onvrijwillig progressief gewichtsverlies als gevolg van een vermindering van de skeletspiermassa (sarcopenie) met of zonder depletie van vetweefsel. Het wordt gekenmerkt door een spectrum van symptomen, variërend van licht gewichtsverlies en anorexia tot ernstig gewichtsverlies, sarcopenie en systemische ontsteking. De incidentie bij kankerpatiënten varieert naar gelang het type kanker, van 80% voor maag- en alvleesklierkankerpatiënten, 50% voor patiënten met long-, dikke darm- of prostaatkanker tot ongeveer 40% voor patiënten met borstkanker of leukemie.

De pathogenese van kankercachexie is multifactorieel en wordt gekenmerkt door een negatieve eiwit- en energiebalans. Skeletspierkatabolisme leidend tot verminderde spiermassa wordt geïnduceerd door zowel tumor- als gastheer- factoren. Vet kan zich ophopen in skeletspieren in de vorm van intramyocellulaire vetdruppeltjes en in de vorm van intermusculaire adipocyten, een fenomeen wat bekend staat als myosteatose. Van cachexie, sarcopenie en myosteatose is aangetoond dat ze een significant negatief effect hebben op de korte- en lange termijn uitkomst en postoperatieve complicaties na een reeks oncologische behandelingen. Waar sarcopenie een indicator is van spiermassa, lijkt myosteatose meer geassocieerd te zijn met een verminderde spierfunctie.

Er zijn verschillende hulpmiddelen ontwikkeld, variërend van eenvoudig te gebruiken vragenlijsten tot complexere computer tomografische (CT) beeldanalyse, om patiënten te identificeren die risico lopen op of symptomen vertonen die verband houden met deze aandoeningen. Voor de doeleinden van dit proefschrift zullen we dieper ingaan op de verschillende methoden die worden gebruikt om cachexie en sarcopenie-geassocieerd chirurgisch risico te identificeren in zowel de klinische als de academische setting. We zullen ook enkele experimentele methoden onderzoeken die niet eerder beschreven zijn.

Risicoscreening

In de oncologie en oncologische chirurgie is screening op ondervoedingsrisico van groot belang. Hiermee kunnen we patiënten identificeren die cachectische eigenschappen vertonen zodat tijdig met een voedingsinterventie kan worden gestart. In *hoofdstuk twee* hebben we een prospectieve observationele studie uitgevoerd om de associatie tussen voedingsrisico's en postoperatieve complicaties te onderzoeken. De resultaten van deze studie toonden aan dat screening op ondervoedingsrisico, in de vorm van de MUST (Malnutrition Universal Screening Tool), een onafhankelijke voorspeller was voor postoperatieve complicaties en verhoogde ernst van complicaties zoals gemeten door de Clavien-Dindo-score bij patiënten die electieve colorectale chirurgie ondergingen (OR 3,82; 95% BI 1,48-9,88) en (OR 2,78; 95% BI 1,13-6,79). Dit werk onderstreepte de relevantie van adequate screening op voedingsrisico's bij electieve colorectale chirurgie.

Computertomografie (CT) -beeldvorming wordt vaak gebruikt in de academische setting voor de detectie van sarcopenie. Deze methode maakt gebruik van CT-beelden op het niveau van de derde lendenwervel (L3) om de dwarsdoorsnede van skeletspier (SM), visceraal vetweefsel (VAT) en onderhuids vetweefsel (SAT) te bepalen, wat goede schattingen oplevert van de totale lichaam SM-, VAT- en SAT-massa. Naast het schatten van massa voor de detectie van sarcopenie kan CT-beeldanalyse ook worden gebruikt voor het meten van spierdichtheid, ook wel SM-RA genoemd. SM-RA wordt beschouwd als een radiologische marker die indicatief is voor myosteatose.

In *hoofdstuk drie* wilden we beoordelen of CT-gemeten sarcopenie en/of myosteatose een toegevoegde waarde hebben naast de MUST voor de voorspelling van postoperatieve complicaties na oncologische colorectale resectie. De resultaten van deze prospectieve observationele studie toonden een hoge prevalentie van sarcopenie aan bij patiënten die een oncologische colorectale resectie ondergingen (52%), maar lieten geen significant verband zien met postoperatieve complicaties. De MUST was univariaat geassocieerd met postoperatieve complicaties ($p<0,05$), myosteatose benaderde significante associatie ($p=0,05$) en sarcopenie vertoonde geen significante associatie bij patiënten die een electieve oncologische colorectale resectie ondergingen ($p=0,59$). Wanneer zowel MUST als myosteatose werden opgenomen in multivariabele analyses vertoonden beide geen significant verband met postoperatieve complicaties ($p=0,12$, OR 4,1, 95%CI 0,7-24,7) en ($p=0,18$, OR 0,5, 95%CI 0,1-1,4). De beperkte cohortgrootte van deze studie vormt een belangrijke beperking en maakte multivariabele analyse ondermaats.

Hoofdstuk twee en drie omvatten de noodzaak van vroege detectie van ondervoeding, sarcopenie en myosteatose door middel van screening op ondervoedingsrisico en CT-metingen van sarcopenie en myosteatose. Ons werk, in lijn met de huidige literatuur, onderstreept het belang van de implementatie van screening in de dagelijkse klinische praktijk. Hoewel onze studie er niet in slaagde een significant verband tussen sarcopenie, myosteatose en een slechtere klinische uitkomst aan te tonen, hebben verschillende onderzoeken sindsdien een sterke associatie onderstreept, waarmee het klinische belang van sarcopenie en myosteatose wordt benadrukt.

Sarcopenie en leverziekte

In het vorige hoofdstuk onderzochten we het belang van screening op ondervoedingsrisico en screening op sarcopenie en myosteatose in een oncologisch-chirurgisch cohort. Naast het belang hiervan in de bovengenoemde setting is sarcopenie een veel voorkomende complicatie van levercirrose en wordt waargenomen bij tot 60% van de patiënten met eindstadium leverziekte (ESLD). Het wordt gekenmerkt door een verminderde adaptieve reactie op vasten als gevolg van een verminderde leverfunctie. De enige effectieve behandeling voor patiënten die lijden aan ESLD is levertransplantatie. De wachttijden voor een transplantatieorgaan kunnen echter lang zijn, waarbij een op de zes patiënten overlijdt terwijl ze wachten op een nieuw orgaan. Verschillende onderzoeken hebben

aangetoond dat sarcopenie gepaard gaat met een hogere wachtlijststerfte en sterfte na transplantatie. De huidige literatuur concentreert zich echter op sarcopenie als maatstaf voor spierverlies, en richt zich niet op myosteatose. Verschillende risicovoorspellers, zoals de Model for End Stage Liver Disease (MELD)-score en de Balance of Risk (BAR)-score, worden gebruikt om de kans op preoperatieve wachtlijststerfte te berekenen. Deze risicoscores missen echter een objectieve parameter die de fysieke toestand van de patiënt weergeeft.

In *hoofdstuk vier* hebben we een retrospectief observationeel onderzoek uitgevoerd naar de voorspellende waarde van myosteatose voor postoperatieve morbiditeit na een levertransplantatie. In deze studie was myosteatose significant geassocieerd met ernstige morbiditeit (Clavien Dindo≥3b) (OR 2.772, 95%CI 1.516-5.066, p=0.001) in multivariabele analyse. Bovendien toonden onze gegevens aan dat met de introductie van myosteatose in de bestaande BAR-score (BAR-myosteatosis-score) een betere voorspelling van 90-dagen morbiditeit en -mortaliteit werd bereikt (AUROC-toename van 0,677 en 0,821 (oorspronkelijke BAR-score)) tot 0,710 en 0,853 (BAR-myosteatose-score). Dit werk draagt bij aan de huidige literatuur door het klinische belang van myosteatose aan te tonen en geeft aan dat myosteatose, indicatief voor een verminderde spierfunctie, van groter klinisch belang zou kunnen zijn dan sarcopenie, wat indicatief is voor spiermassa in een levertransplantatiecohort.

Sarcopenie, myosteatose en longontsteking

In het vorige hoofdstuk werd het belang van myosteatose (verminderde spierfunctie) boven sarcopenie (spiermassa) naar voren gebracht. Postoperatieve pneumonie is een belangrijke oorzaak van postoperatieve morbiditeit en mortaliteit. In *hoofdstuk vijf* hebben we een prospectief observationeel onderzoek uitgevoerd naar het verband tussen myosteatose ter hoogte van de derde lumbale (L3) of vierde borstwervel (T4) en de incidentie van postoperatieve pneumonie. We vonden dat L3- en T4-myosteatose beide in gelijke mate geassocieerd waren met postoperatieve pneumonie in een multivariabel model (OR 3,65, 95% BI 1,41-9,49, p<0,01) en (OR 3,22, 95% BI 1,20-8,61, p=0,02).

Het onderzoek naar pulmonale morbiditeit leidde ons naar de analyse van het middenrif. Interessant is dat er zeer weinig bekend is over de functie van het middenrif in de context van sarcopenie en myosteatose, of hoe de functie ervan wordt beïnvloed door abdominale chirurgie. Daarom ontwierpen we een prospectieve observationele studie, beschreven in *hoofdstuk zes*, die de functie van het middenrif tijdens de perioperatieve periode onderzoekt met behulp van echografie. In deze momenteel nog lopende studie evalueren we verschillen in middenriffunctie en herstel tussen sarcopene en niet-sarcopene patiënten die een grote leverresectie ondergaan. De resultaten van deze prospectieve observationele studie zullen bijdragen aan het begrijpen van de rol van het diafragma in het optreden van pulmonale morbiditeit na grote leverresecties, en mogelijk bijdragen aan het identificeren van patiënten die risico lopen op longontstekingen.

Radiomics

In voorgaande hoofdstukken werden CT-beelden gebruikt om informatie over spiermassa, spierdichtheid en intra-abdominaal vet te verkrijgen. Hiermee werd een kwantificering van de spiermassa van het hele lichaam (sarcopenie) en spierdichtheid (myosteatose) gedaan (conventionele beeldanalyse). Deze methode wordt beschouwd als de gouden standaard voor analyse van sarcopenie en myosteatose met behulp van CT-beelden.

Radiomics-analyse haalt grote hoeveelheden gegevens uit medische beelden. Waar conventionele beeldanalyse zich richt op een handvol beeldeigenschappen die door medische deskundigen worden geïnterpreteerd, extraheert radiomics-analyse honderden en soms duizenden beeldeigenschappen (radiomics-kenmerken). Dit levert grote hoeveelheden kwantitatieve beelddata op die op hun beurt worden gesorteerd met behulp van complexe data-analyse. Associaties tussen beeldgegevens die niet met het blote oog te zien zijn en klinische uitkomst worden vervolgens geanalyseerd. Radiomics-analyse vindt zijn oorsprong in de beeldvorming van tumoren, waarbij aanvullende tumoreigenschappen met succes uit CT-beelden gewonnen zijn. Deze tumoreigenschappen bleken van toegevoegde waarde ten opzichte van conventionele interpretatie door medische deskundigen.

In *hoofdstuk zeven* onderzochten we de implementatie van nieuwe radiomics-benaderingen op het gebied van beeldvorming van de lichaamssamenstelling. We onderzochten retrospectief of de kwantificering van grote hoeveelheden beeldvormingsgegevens over lichaamssamenstelling een meerwaarde kan bieden voor de identificatie van patiënten met een risico op verminderde overleving na oncologische pancreasresectie. Ons onderzoek liet zien dat het haalbaar is om een radiomicsbenadering te implementeren voor beeldvorming van de lichaamssamenstelling. We hebben drie modellen gecreëerd; een Radiomics-model (R-score) dat alleen radiomics-kenmerken bevat, een klinisch model (C-score) dat alleen klinische gegevens bevat en een conventioneel lichaamssamenstellingsmodel (B-score) dat spiermassa, spierdichtheid en vetmassa bevat. Deze modellen werden vervolgens vergeleken in een trainings- en validatiecohort. De gemiddelde Harrell-concordantie-indices voor modellen van de totale overlevingstijd waren het hoogst voor het radiomics-model of de R-score (0,61, 95% BI 0,56 – 0,65, p<0,001), gevolgd door het klinische model met alleen klinische gegevens of de C-score (0,59, 95% BI 0,55 - 0,63, p<0,001) en tot slot de conventionele lichaamssamenstellingsscore, B-score (0,55, 95% BI 0,50 - 0,60, p=0,03). Deze gegevens geven aan dat radiomics-kenmerken een vergelijkbare of licht verbeterde voorspellende waarde lijken te hebben in vergelijking met conventionele lichaamssamenstellingsvariabelen. Bovendien laten onze gegevens zien dat compartimenten van buikvetweefsel, skeletspieren en onderhuids vetweefsel allemaal radiografische kenmerken of beeldgegevens bevatten die voorspellende informatie lijken te bevatten voor de algehele overleving. Deze resultaten bleken echter niet allemaal significant in het onafhankelijke validatiecohort.

CONCLUSIE

Het werk dat in dit proefschrift naar voren wordt gebracht illustreert het belang van een holistische benadering van patiëntenzorg. Het benadrukt de noodzaak om de meest kwetsbare patiënten te identificeren, die het grootste risico met zich meebrengen. Onze momenteel lopende studie naar middenriffunctie zal mogelijk bijdragen aan de identificatie van patiënten die risico lopen op postoperatieve longontsteking. Dit zou in de toekomst kunnen leiden tot een vroege preconditionering van patiënten en daarmee een verbetering van de postoperatieve longfunctie. Preconditionering van patiënten zou ook van toegevoegde waarde kunnen zijn voor sarcopene en myosteatotische patiënten die een levertransplantatie ondergaan. Evaluatie van dergelijke behandelingen zou de volgende stap moeten zijn in het verbeteren van de uitkomsten van onze meest kwetsbare patiënten. Ons werk op het gebied van radiomics-analyse vormt een nieuwe benadering van beeldvorming van de lichaamssamenstelling, en kan bijdragen aan de discussie of radiomics-analyse waardevol is en of het een plaats kan hebben in de academische en/of dagelijkse klinische praktijk.

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Tina, jij en ik schreven beide een proefschrift naast ons dagelijks werk in de kliniek. Jij als assistent in Nederland, als rising star in de colorectale robot chirurgie. Ik als assistent in Duitsland, een ander systeem, een andere cultuur. Onze avonden, diners samen waren altijd een welkome afleiding van de dagelijkse stress en hectiek.

Marijke. Mijn Duitse vriendin die in Nederland werd opgeleid waar ik als Nederlander in Duitsland werd opgeleid. Nu werk jij als pediatrische chirurg in Duitsland waar ik in Nederland fellow ben… gek hoe het leven soms loopt. Ook jij was altijd een steun en toeverlaat in de dagelijkse hectiek, en in het begin van mijn tijd in Duitsland, mijn privé Duits tutor.

Aan mijn levenspartner, en verloofde. Lieve Victoria jij bent er altijd voor mij. Je hebt altijd begrip voor mijn ambitieuze carrière, onregelmatige leven. Bij jou valt alle stress van het dagelijkse leven weg.

Mijn paranimfen, Ruud en Marius. Marius, lieve Mar. Hoe stressvol, chaotisch, druk, frustrerend, veeleisend het leven ook is, jij bent er altijd. Als paranimf heb je me samen met Ruud figuurlijk over de eindstreep getrokken. Ik ben blij dat ik dit samen met jou mag ervaren.

Lieve Ruud. Recent heb jij ook de vreugde van het afsluiten van je proefschrift mogen ervaren. Tijdens onze gezamenlijke tijd als Phd-candidates filosofeerden wij menig avond over de zin des levens, en het soms als een onoverkomelijke brok werk wat we voor ons hadden, het proefschrift. Wij zullen altijd iets vinden om over te filosoferen…