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Defining clinically relevant myosteatosis in patients with liver cirrhosis

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Defining clinically relevant myosteatosis in patients with liver cirrhosis

Journal Pre-proof

Current cut-offs are non-liver-specific and BMI-based.

CANADIAN COHORT

n=863 pts



Monocentric, retrospective
validation cohort

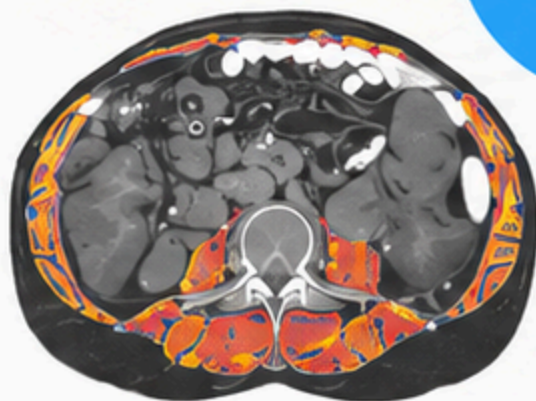
ITALIAN COHORT

n=433 pts



Multicentric, prospective
derivation cohort

1296 pts



L3 / L3-L4
CT-scan

Aim: define a cirrhosis-specific cut-off
linked to 1-year mortality.

METHODS

STEP 1

Interobserver agreement
across 60 slices

ICC > 0.97

STEP 2

Validation of Ebadi et al. cut-offs

Limited prognostic consistency
across cohorts

STEP 3

New cut-off derived in Italy
and validated in Canada

ROC analysis

NEW CUTOFF



No sex- or BMI-
based stratification

1-year mortality

Adjusted hazard ratio (aHR, 95% CI)

Italian cohort

1.83 (1.03–3.24)

Canadian cohort

1.63 (1.25–2.11)

0.5 1 2 4
Adjusted hazard ratio (aHR)

WHAT IS NEW:

- Disease-specific cut-off
- No sex- or BMI-based stratification

**≤26 CT scan HU is reproducible and
linked to mortality in cirrhosis.**

Title: Defining clinically relevant myosteatorsis in patients with liver cirrhosis

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Michele Campigotto—contributed to patient enrolment, data collection, and data curation. All authors critically reviewed the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

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Highlights

- Interobserver error in the assessment of myosteatosi in advanced liver disease is unknown, nor has a specific cutoff been agreed upon
- We found that muscle attenuation assessment, as a surrogate for myosteatosi, is highly reproducible across independent centres.
- A cut-off of ≤ 26 HU Hounsfield units, independent of sex and BMI, allows detecting patients with similar mortality risk across independent centres.
- If further prospectively validated, this cut-off would allow reproducible patient risk-classification across cohorts with different baseline risk.

Impact and implications.

Myosteatosi is an important prognostic feature in cirrhosis, but its clinical use has been limited by the lack of disease-specific criteria. In two independent cohorts, a muscle attenuation threshold of ≤ 26 Hounsfield units consistently identified patients at increased 1-year mortality risk, irrespective of sex and body mass index. This simple and reproducible cut-off could support more uniform risk stratification in clinical practice and research when abdominal CT imaging is available, and may facilitate patient selection for future interventional studies. Further prospective validation will help define its role in routine care.

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ABSTRACT

Background: Myosteatorsis is increasingly recognized as an indicator of high mortality risk in cirrhosis. However, no validated criteria to define this skeletal muscle alteration are currently available for clinical practice. We aimed to validate the recently proposed University of Alberta criteria and to assess their association with mortality.

Methods: Individual patient data analysis combining two cirrhosis cohorts from Italy and Canada. Muscle attenuation and skeletal muscle index were assessed on CT scans at the L3 or L3–L4 level using a standardized protocol. Interobserver agreement was evaluated using intraclass correlation coefficients. Since the performance of the University of Alberta criteria in the Italian cohort was unsatisfactory, we used ROC analysis to derive a new attenuation threshold in the Italian cohort and validated it in the Canadian cohort. The association of the new cutoff for myosteatorsis and its combination with sarcopenia and 1-year mortality was assessed by a proportional hazards model for competing risks, with liver transplantation as a competing event.

Results. A total of 1,296 patients were included, with substantial differences in disease severity and baseline mortality rates between the cohorts. Interobserver agreement for muscle attenuation and skeletal muscle index was excellent (ICC >0.97). ROC analysis in the Italian cohort identified a muscle attenuation threshold of 26 Hounsfield units (HU) as the most discriminant cut-off for mortality risk, independent of sex and BMI. Patients' reclassification according to this threshold showed a sHR of 1.83 (95% CI 1.03–3.24; $p=0.038$) in the Italian and 1.63 (95%CI 1.25-2.11; $p<0.001$) in the Canadian cohort. Corresponding figures for myosteatorsis combined with sarcopenia were 1.35 (95% CI, 1.07-1.71; $p=0.01$) and 1.32 (95% CI, 1.20-1.47; $p<0.001$), respectively.

Conclusions: A muscle attenuation cut-off of HU ≤ 26 allows reproducible detection of myosteatorsis in cirrhosis, across populations with different baseline disease severity.

Introduction

Myosteatosis is a clinically relevant alteration of skeletal muscle characterized by intramuscular fat infiltration, resulting in impaired muscle quality and function¹. It likely represents an early stage of progressive muscle deterioration, in which lipid accumulation promotes metabolic and pro-inflammatory changes that may ultimately lead to sarcopenia²⁻⁴. Although common in aging, myosteatosis is frequently observed in chronic inflammatory conditions such as liver cirrhosis and is closely associated with visceral obesity, insulin resistance, and type 2 diabetes mellitus⁵. In chronic liver disease, hyperammonemia and insulin resistance synergistically impair mitochondrial lipid oxidation and promote fibrotic and fatty skeletal muscle remodeling^{6,7}. Clinically, myosteatosis has been associated with reduced transplant-free survival, increased risk of hepatic encephalopathy, prolonged hospitalization, and post-transplant complications⁸⁻¹². However, most available evidence relies on heterogeneous, non-disease-specific cut-offs—largely derived from oncological populations and frequently incorporating BMI-based stratification¹³—resulting in substantial risk of misclassification, particularly in cirrhosis where body weight assessment is unreliable. In this context, our recent prospective multicentre study demonstrated that the coexistence of myosteatosis and sarcopenia confers a particularly poor prognosis across different stages of cirrhosis^{14,15}.

Muscle attenuation evaluated by CT is a highly accurate and validated method for evaluating fat within the muscle¹⁶. CT can distinguish fat from muscle based on tissue attenuation, which is a function of tissue density and chemical composition. In this regard, the mean attenuation of muscle measured by CT reflects the lipid content determined by biochemical and histological analysis¹⁷. These radiodensity maps have been heavily validated and show strong, direct correlations with actual fat content verified by muscle biopsies and MRI¹⁸. Currently the detection of myosteatosis is based on the assessment of muscle attenuation at the L3 vertebral level by computed tomography (CT), measured as Hounsfield Units (HU), or by magnetic resonance imaging (MRI). While MRI is more accurate and eliminates the risk of overestimation in the presence of fluid retention¹⁹, CT is more widely available and therefore remains the most commonly used and better evaluated imaging technique in clinical practice²⁰.

In 2022, Ebadi et al at the University of Alberta. proposed alternative cut-offs (<33 HU for men and <28 HU for women), which allowed detection of myosteatorosis independently of BMI and were more strongly associated with mortality in a large retrospective cohort of patients with cirrhosis¹⁵. A recent systematic review and meta-analysis⁸, in light of the substantial heterogeneity of the proposed cut-off values and the lack of specific validation in cirrhosis, underscored the need for a consensus on optimal criteria to assess myosteatorosis in patients with chronic liver disease. This study aimed to validate Alberta's cut-offs and, in case of unsatisfactory validation, to identify new thresholds for clinically significant myosteatorosis.

Methods.

Study design.

This cohort study used individual participant data (IPD) derived from two independent studies assessing sarcopenia and myosteatorosis in cirrhosis. Of the two cohorts, one was recruited at the University of Alberta (Canada)¹⁵, and the other at the Sapienza University of Rome (Italy)¹⁴ and are referred to as the Canadian and the Italian cohorts, respectively, throughout the text. Data from the two cohorts were collated in a unique, anonymized database. After verifying interobserver variability in muscle surface and radio-intensity measurements across the two recruiting centers, Alberta's criteria to define myosteatorosis were validated in the Rome cohort. The study was registered on *ClinicalTrials.gov* (<https://clinicaltrials.gov/study/NCT06593015>), and the corresponding protocol was published in March 2025²¹. Since the performance of University of Alberta criteria in the Italian cohort was not satisfactory, a post-hoc search for a more efficient muscle attenuation threshold for myosteatorosis was subsequently planned. Data collection took place between January and March 2025, and statistical analyses were completed by July 2025.

Patients

Patients with liver cirrhosis were enrolled at the time of their CT scan muscle assessment in both studies: between January 2000 and August 2021 in the Canadian and between January 2019 and December 2021 in the Italian cohorts. Inclusion and

exclusion criteria differed partly between the two cohorts. The Canadian cohort included adults with cirrhosis evaluated for liver transplantation (LT), while patients with multi-organ transplantation or liver re-transplantation were excluded. The Italian cohort included patients with cirrhosis at any stage, excluding those with a history of, or listed for LT, those with hepatocellular carcinoma (HCC), concomitant neuromuscular disease, current malignancy, history of serious extrahepatic disease, or HIV infection.

Muscle assessment in the original studies

In both cohorts, muscle mass and quality were assessed using CT imaging analyzed by the Tomovision software Version 4.2 (Montreal) (Figure 1). All CT slices evaluated were pre-selected and obtained from the no contrast phase. For the Canadian cohort, measurements were performed at the L3 vertebral level, while in the Italian cohort, the L3-L4 slice was used. HU thresholds for skeletal muscle were set between -29 and +150 HU. In the Canadian cohort, sex-specific cut-offs for myosteatosis were applied: <33 HU for men and <28 HU for women¹⁵. In the Italian cohort, myosteatosis was defined based on BMI: <41 HU for patients with a BMI <24.9 kg/m², and <33 HU for those with a BMI ≥25 kg/m²¹³. Muscle cross-sectional area was indexed to height (cm²/m²) to obtain the skeletal muscle index (SMI). In both cohorts, sarcopenia was defined using validated SMI thresholds: <50 cm²/m² in men and <39 cm²/m² in women²² and patients were classified according to the muscle abnormality phenotype into four categories, as proposed by Ebadi et al.¹⁵: no myosteatosis/no sarcopenia (no muscle changes); isolated myosteatosis; isolated sarcopenia; sarco-myosteatosis (coexistence of both sarcopenia and myosteatosis).

Follow-Up and Outcomes

The primary outcome was overall mortality, with LT considered a competing event. The reported length of follow-up (mean ± standard deviation) in the two original studies was 738 ± 1074 days for the Canadian cohort and 450±259 in the Italian one. Therefore, for the present study, we used 365-day outcomes for more homogeneous estimates across the two cohorts.

Data collection and handling.

Anonymized data from the two cohorts were collected according to an agreed-upon data collection form after adequate pilot testing²¹. Collected data included clinical history, baseline patient characteristics and CT-scan muscle assessment, follow-up events, and final status. Data congruence with the requested information was independently checked by two authors (SDC and GD), and data consistency and accuracy were further checked by cross-tabulation; any discrepancy was resolved by asking the physician responsible for the relevant data record. Missing information was retrieved from clinical records whenever possible.

Interobserver variability in CT scan muscle assessment.

To assess whether the assessment of muscle alterations was homogeneous across the two centers where the original studies were performed, the interobserver variability in CT scan muscle assessment was investigated. To this aim, a random sample of 60 CT scans, 30 from Rome and 30 from Alberta, were independently and blindly assessed by two expert physicians (M. M. from Alberta and S.D.C. from Rome) to measure mean muscle attenuation, muscle cross-sectional area, and SMI. The analysis of interobserver variability was then blindly performed.

Validation of University of Alberta cutoffs for myosteatorsis

Patients in the Italian cohort were classified with myosteatorsis according to Alberta's cutoffs¹⁵ and the above-reported classification of muscle damage: no damage, isolated myosteatorsis, isolated sarcopenia, sarco-myosteatorsis. The clinical impact of these muscle damage phenotypes on mortality was then assessed by the Fine and Gray proportional hazards model for competing risks, with LT considered as a competing event. The SHR of myosteatorsis for mortality in the Italian cohort adjusted for the same covariates as in the Alberta study was compared to that reported by Alberta¹⁶. The muscle attenuation cutoffs for detecting myosteatorsis and the proposed classification were considered satisfactorily valid if the differences between the SHRs in Alberta study and in the Italian cohort were not statistically significant, with overlapping of at least two-

thirds of the 95% CIs.

Exploring radio-intensity cutoffs with better performance to predict mortality

In a post-hoc analysis, we explored whether a different cutoff of muscle attenuation to define myosteatosis could have a higher association with mortality than those proposed by Ebadi et al.¹⁵. The analysis was performed in the Italian cohort and validated in the Canadian one.

Ethical considerations.

This study included patients who had provided informed consent to participate in each of the two studies, and both studies were approved by the local ethics committee at the University of Alberta and at the University Sapienza in Rome. All data for analysis was strictly anonymized.

Statistical Analysis

Baseline characteristics were summarized as proportions and means with standard deviation (SD). Differences between groups were assessed using the Chi-square or Student's *t*-test, as appropriate. Interobserver variability in the assessment of muscle attenuation measured as HU and muscle surface measured as SMI was assessed using intraclass correlation coefficients (ICCs). Both individual and average ICCs were calculated by a one-way random-effects model to quantify the degree of agreement between observers²³. ICC analyses were stratified by sex and cohort. Agreement was further explored using graphical methods, including box plots and linear regression analyses.

Survival was analyzed using the Kaplan–Meier method²⁴, with subgroup differences assessed by the log-rank test. Time zero was the date of the CT scan assessment of muscle damage. The cumulative incidence function (CIF) of death was estimated using competing risks analysis²⁵, with liver transplant (LT) as a competing event.

Differences between groups were assessed using Gray's test by Lambert's procedure in STATA²⁶, with 95% CI expressing uncertainty. To assess the adjusted SHR (aSHR) of muscle damage on mortality, the proportional hazards model for competing risks by

Fine and Grey²⁷ was used. Adjusting variables were the same used by Ebadi et al¹⁵: age, sex, etiology of cirrhosis, albumin, MELD, ascites, sodium, hepatic encephalopathy, actual or prior variceal bleeding, diabetes, BMI, myosteatosis, and sarcopenia. Muscle abnormality phenotype (coded as: 0= no myosteatosis, no sarcopenia; 1=sarcopenia, no myosteatosis; 2= myosteatosis, no sarcopenia; 3= myosteatosis and sarcopenia) was included in separate, sensitivity analyses to avoid redundancy. Analyses were performed by a backward procedure selecting variables according to clinical relevance and statistical significance at 0.10 level. Models including the Italian cohort were performed by including the covariates significant in the Canadian cohort, as reported in the original article and forcing myosteatosis and sarcopenia.

To validate Alberta's criteria to define the muscle abnormality phenotype, we compared the aSHR of the corresponding variable computed in the Italian cohort with that computed in the Canadian cohort. We accepted as satisfactory validation of the criteria if the two aSHR were not significantly different as indicated by overlapping 95%CI.

In a post-hoc analysis, we explored whether a different radio-intensity cutoff for myosteatosis could allow a reclassification of muscle abnormality phenotype with higher association with mortality. After testing the two cohorts for normal distribution of muscle radio-intensity, we performed ROC analyses of radio-intensity for mortality, stratified by sex and assessed the best cut-point by the Liu test²⁸ in the Italian cohort. The selected cut-points were then used for patient reclassification. The new risk classification derived in the Italian cohort by the same multivariable analyses as above reported was internally validated using 500 bootstrap resampling to achieve shrunken coefficients and reduce optimism. The new risk classification was then externally validated in the Canadian cohort. To formally evaluate whether the prognostic effect of the proposed muscle damage classification was consistent across the derivation and validation cohorts, a Fine–Gray competing-risk regression model was fitted in the pooled dataset including an interaction term between cohort and muscle damage classification. A non-significant global interaction test was considered evidence that the prognostic effect did not differ between cohorts. The incremental discrimination of

the new classification was assessed by comparing Harrell's concordance index²⁹ and time-dependent area under the ROC curve (AUC) for censored survival data³⁰ of the final multivariable model, including and excluding the relevant covariate. Incremental discrimination was quantified as the difference in C-index between models, with 95% confidence intervals estimated by bootstrap resampling with 1000 repetitions.

Results.

Patients. The study population consisted of 1,296 patients: 863 (66.6%) from the Canadian and 433 from the Italian cohort. Patients' characteristics are shown in Table 1. The two cohorts were significantly different with regard to disease etiology and severity. Among the most important differences in Italian vs Canadian cohorts, etiology was from alcohol in 40% vs 25%, and from HCV in 16% vs 39% respectively (both differences $p < 0.001$); prevalence of hepatic encephalopathy at inclusion was 20% vs 37% and mean MELD score ($\pm$ SD) was 12.9 ($\pm$ 4.9) vs 15 ($\pm$ 8) (both < 0.001); also, radiologic muscular parameters were in general more compromised in the Italian cohort. Overall, patient characteristics at inclusion showed a more advanced disease in the Canadian cohort with corresponding significantly higher incidence rate/patient-year of death (0.20 vs 0.12, $p < 0.0001$) and liver transplant (0.20 vs 0.097, $p < 0.0001$). These important case-mix differences suggest that the validation results of this study should be interpreted more in the sense of transportability than reproducibility³¹.

Interobserver agreement.

Individual measurements of muscle attenuation and muscle area independently observed at the two participating centers in the random sample of 60 patients were almost fully overlapping (Figure 2). Individual ICCs for the measurement of muscle attenuation in HU were 0.98 for individual values and 0.99 for the average. Concordance remained > 0.98 even when stratified by sex (Supplementary Table 1). Similar Concordance was found also for assessment of muscle area, indicating that assessment of myosteatosis and sarcopenia, based on predefined threshold values of muscle attenuation and surface on CT-scan slices at the same level, may be expected to result in homogeneous patient classification across different centers.

Validation of Alberta's myosteatorsis criteria in the Italian Cohort

In the original study of the Italian cohort¹⁴ 302 patients were classified with and 131 without myosteatorsis according to previously published criteria¹³. When applying Alberta's criteria, 96 of the 302 with- were reclassified as without myosteatorsis, while the classification of sarcopenia remained unchanged because it was based on the same definition in the two cohorts. The reclassification of patients in the Italian cohort according to Alberta's criteria for the two variables myosteatorsis and sarco-myosteatorsis (muscle change phenotype) is shown in Table 2.

After reclassification of the Italian cohort for myosteatorsis by Alberta's criteria, the SHR of myosteatorsis for death adjusted by the same covariates as in Alberta's study, was 3.32 (95%CI 1.67-6.61) compared to 1.47 (95%CI 1.17-1.84) in the Canadian cohort (Table 3). The more than double aSHR in the Italian cohort, with almost nil CI overlap, was not considered an acceptable validation of Alberta's criteria. CIF curves of death (LT competing) in the Italian cohort according to reclassified muscle damage showed a slightly higher risk of death across all the categories of muscle damage, compared to the previous classification (Figure 3), with the difference between CIF of death in patients with myosteatorsis plus sarcopenia now significantly higher than that of patients free of muscle damage.

Search and validation of new muscle attenuation cutoffs for myosteatorsis.

After testing the distribution of muscle attenuation (Supplementary Figure 1) for normality, we performed ROC analyses for mortality in the Italian cohort and selected the best cut-point for a new definition of myosteatorsis. Since the c-statistics were not significantly different according to sex (0.70, 95% CI 0.61-0.79 in males; 0.67, 95% CI 0.55-0.78 in females; $p=0.65$), we selected the best cutoff-point by the Liu test²⁸ in the whole Italian cohort, which yielded a value of 26 HU. By using this new cutoff, we reclassified myosteatorsis as yes if muscle radio-intensity was ≤ 26 HU and no for values > 26 (Table 4). The association with mortality risk of this reclassification

derived from the Italian cohort was then validated in the Canadian cohort. The adjusted SHR of myosteatorsis for death was 1.83 (95% CI 1.03-3.24) in the *derivation* Italian cohort and 1.63(95% CI 1.25-2.11) in the *validation* Canadian cohort (Table 5). The corresponding SHR for the muscle damage phenotype, coded as no damage, isolated sarcopenia, isolated myosteatorsis, and sarcopenia plus myosteatorsis, was 1.35 (95% CI 1.07–1.71) in the Italian cohort, and was 1.07 (95% CI 1.02–1.12) after a shrinkage bootstrap analysis with 500 resamples (Supplementary table 2). In the validation cohort, the SHR for death associated with the muscle damage phenotype defined by the new radiodensity cut-off was 1.32 (95% CI 1.20–1.47) (Table 5), and the observed and expected CIFs of death according to the new muscle damage phenotype classification were very similar.(Figure 4). In a pooled analysis, no significant interaction was found between cohort (Rome vs Alberta) and the proposed muscle damage classification (global interaction test: $\chi^2=2.64$, $df=3$, $P=0.451$), indicating that the prognostic effect of the four-category classification was consistent across the derivation and external validation cohorts (Supplementary table 3). In the Canadian cohort, time-dependent AUROC analysis of the final model derived in the Roma cohort (table 5), including and respectively not including the muscle-damage phenotype, consistently showed a small (although significant) incremental predictive value of the new classification across the one-year observation period (Figure 5).

Discussion.

This is the first study specifically designed to define a liver disease-oriented cut-off for clinically relevant myosteatorsis in patients with cirrhosis, based on two large independent cohorts from Italy¹⁴ and Canada¹⁵. We first showed that the muscle radio-intensity and muscle surface assessment based on a pre-specified level of CT-scan slices is highly reproducible across independent assessors at independent centers when using the same software. The intra-class concordance for assessments at the two centers was higher than 98% for the assessments of both parameters,

ensuring that patients' classification based on predefined cutoffs of muscle surface and radio-intensity was reliable and reproducible across different centers. Although this result might have been predicted based on the automated reading of CT-scan parameters, there is an "assessor dependent" part in the overall assessment, because it is the assessor who selects the muscle area where the CT-scan software makes the relevant measurement.

We then assessed the validity of Alberta's cutoffs for myosteatorsis, derived from the Canadian cohort¹⁵ in the Italian cohort¹⁴. Although the proposed cutoffs effectively allowed to identify patients classified as having myosteatorsis at significantly higher risk of death compared to patients without myosteatorsis, the relevant adjusted SHR for death in the Italian cohort was more than double that in the Canadian cohort. This result suggests that the cutoffs proposed by Ebadi et al¹⁵ to classify myosteatorsis are not likely generalizable with regard to the selection of patients with homogeneous death risk.

We therefore investigated, in a post-hoc analysis, the possibility of detecting cutoffs for the definition of myosteatorsis, allowing for homogeneous patient risk-stratification across independent centers. To this aim, the Italian cohort acted as the derivation cohort and the Canadian as the validation cohort. This analysis showed that defining myosteatorsis based on a radio-intensity cutoff of 26 HU independent of sex and of BMI allows the detection of patients at a similar aSHR of death across the derivation (Italian) and validation (Canadian) centers: respectively 1.84 (CI 1.03-3.24) and 1.63 (CI 1.25-2.11). Importantly, this also resulted in very similar aSHR of muscle damage phenotype, classified as no damage, sarcopenia only, myosteatorsis only, and myosteatorsis plus sarcopenia: 1.35 (CI 1.07-1.71) in the Italian and 1.32(CI 1.20-1.47) in the Canadian cohorts. Moreover, the addition of the new classification to the baseline model including MELD score and hepatic encephalopathy yielded a small but statistically significant increase in discriminative performance for mortality prediction (Figure 5). This result suggests that this muscle damage classification in cirrhosis may be used in clinical practice and research, allowing a homogeneous risk classification of patients across different centers. The importance of this result is underlined by the observation that, although some studies have confirmed the association between

myosteatosi and survival, the majority of these studies have applied thresholds derived from oncological populations¹³, which are not liver disease-specific and often incorporate BMI-based stratification—an approach that is challenging in cirrhosis due to increased water retention and unreliable assessment of body weight³².

Other studies have utilized cutoffs derived from ROC curves; however, a common disease-specific cutoff has not been identified. This is further highlighted by a recent meta-analysis including 14 cohort studies and 4287 showing a pooled risk ratio of 1.94 (CI 1.61-2.34) for transplant-free survival in patients compared to those without myosteatosi³³. However, the authors concluded that a major limitation of their meta-analysis was the important heterogeneity of the radio-intensity cutoff values used to define myosteatosi. Another meta-analysis⁸ reported a mean prevalence of myosteatosi of approximately 50%, with rates exceeding 80% in some cohorts, a figure that is unlikely to reflect a clinically meaningful risk stratification.

In line with these results, in our previous prospective multicenter study¹⁴, myosteatosi was present in nearly 70% of patients. In this study, although muscle attenuation as a continuous variable was independently associated with mortality, the categorical diagnosis of myosteatosi lost significance when adjusted for clinical and biochemical prognostic indicators, suggesting substantial misclassification when using conventional cut-offs. If further independently validated, the new cutoff of 26 HU shown in the present study might result in a generalizable unified criterion to classify myosteatosi for homogeneous patient risk stratification both for clinical practice and research.

The main strengths of our study lie in the pre-established protocol²¹, inclusion of two large, independent cohorts, from two different countries: the distinct disease severity, etiological distribution, and clinical management of the two cohorts point to a potential transportability of the results³¹. Additional strengths include the use of individual patient data, rigorous data harmonization across centers, and assessment of interobserver variability, which demonstrated excellent reproducibility of muscle attenuation measurements. Furthermore, the new cut-off was derived independently of BMI, which is frequently unreliable or confounded in patients with cirrhosis, thereby improving clinical applicability.

Several limitations should also be acknowledged. First, despite careful harmonization, the retrospective nature of the post hoc analysis to detect the new cutoff may have introduced residual confounding, and causality cannot be inferred, even in front of his highly satisfactory independent validation. Second, CT scans were acquired for clinical indications and not according to a standardized imaging protocol, which may have contributed to center-specific heterogeneity, although interobserver agreement was excellent and HU distributions were comparable. Finally, while CT-derived muscle attenuation is widely available, it does not capture functional or molecular aspects of muscle quality, and future studies integrating imaging with functional, inflammatory, and metabolic biomarkers are warranted.

In conclusion, this study establishes a novel, disease-specific cut-off for myosteatorsis in cirrhosis. A muscle attenuation threshold of ≤ 26 Hounsfield Units identifies a clinically meaningful subgroup of patients at increased mortality risk, independent of sex and BMI. Adoption of this liver disease-specific definition may improve prognostic stratification, reduce misclassification, and support the integration of muscle quality assessment into routine care and future interventional studies in cirrhosis.

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FIGURES.

Figure 1. CT images quantify muscle radiodensity in patients with cirrhosis.

Comparison of two patients with cirrhosis and similar skeletal muscle index. A) Patient

with low mean muscle radiodensity (24 HU) or myosteatosi, and B) Patient with normal muscle radiodensity (51 HU, no myosteatosi)

Figure 2. Individual patient muscle area and radio-intensity in a 60-patient random sample (30 per each cohort) as measured by 2 independent raters (1 per each center)

Figure 3. Outcome in the Italian cohort after muscle damage reclassification according to Alberta's criteria (left panel), compared to the previous classification (right panel). 1-year cumulative incidence functions are reported in the boxes with 95% CI in parenthesis. Numbers below the abscissa are patients at risk.

Figure 4. Observed (left panel) and expected CIF of death (right panel), according to the new muscle damage phenotype classification in the Canadian cohort. 1-year cumulative incidence functions are reported in the boxes with 95% CI in parenthesis. Numbers below the abscissa are patients at risk.

Figure 5. Incremental predictive value of the muscle damage phenotype for mortality in the Alberta cohort of the new classification by the model derived in the Roma cohort (table 5). Left panel: time dependent AUROCs of the final multivariable model derived in the Roma cohort including and, respectively not including the new muscle damage phenotype, indicated as sarcomyosteatosi. Right panel. 1-year C-index difference for mortality of the final multi-variable model including and, respectively not including the new muscle damage phenotype.

Table 1. Patients' characteristics

	Canadian cohort		Italian cohort		Difference (95%CI)
	Missing	Complete info*	Missing	Complete info*	
Patients		855		433	-
Demographics					
Age, yrs	6	56.3 (8.7)	-	57.1 (8.9)	3.8(2.8-4.8)
Sex M	-	535 (62.5)	-	308 (71.1)	8.5(3.2-13.9)
BMI	1	27.2 (5.6)	1	27.8 (5.5)	
Etiology					
Alcohol	-	215 (25.1)	-	187 (43.2)	18.0(12.5-23.5)
HCV	-	336 (39.3)	-	68 (15.7)	23.6(18.9-28.3)
HBV	-	55 (6.4)	-	19 (4.4)	2.0(0.05-4.5)
MASH	-	171 (20.0)	-	39 (9.2)	11.0(7.2-14.8)
Autoimmune/Biliary disease	-	71 (8.3)	-	9 (2.1)	6.2(3.9-8.5)
Combined etiologies	-	0		48(11.1)	11.1(8.1-14.0)
Others or undefined	-	55(12.8)	-	7 (0.8)	11.9(8.7-15.1)
Clinical and laboratory parameters					
Hepatic encephalopathy	-	318 (37.2)	-	86 (19.9)	
MELD score	21	15 (8)	-	12.9 (4.9)	1.5(0.7-2.3)
Sodium,	37	135.8 (4.8)	3	138.0 (4.1)	2.4(1.8-3.0)
Albumin, gr/L	28	29.6 (7.1)	1	35.8 (7.2)	6.3(5.5-7.2)
CT parameters and clinical classification#					
SMI	-	48.9 (9.8)	-	50.3(10.9)	1.4(0.22-2.6)
Muscle Attenuation	-	34.4 (9.0)	-	31.6(8.4)	2.8(1.8-3.8)
VAT	1	36.6 (27.4)	-	52.4 (31.5)	15.7 (12.3-19.1)
SAT	5	60.2 (40.7)	1	69.3 (43.3)	8.9(4.1-13.7)
RSAT	24	86.3 (16.1)	-	69.3 (43.3)	5.2 (3.4-7.1)
Muscle changes as reported in the original studies					
No muscle changes	-	383 (44.8)	-	96 (22.2)	22.6(17.5-27.8)
Isolated-sarcopenia	-	177 (20.7)	-	35 (8.1)	12.6(8.9-16.4)
Isolated-myosteatosis	-	147 (17.2)	-	166 (38.3)	21.1(15.9-26.4)
Sarco-myosteatosis	-	148 (17.3)	-	136 (31.4)	14.1(9.0-19.2)
outcome events					
Follow-up, days	-	738(±1075)	-	450(±259)	288(185-391)
Total patients-year	-	1726	-	533	-
Total deaths	-	366 (42.8)	11	54 (12.8)	30.0(25.4-34.6)
Deaths/patient-year	-	0.20	11	0.12	-
Total LT	-	349 (40.8)	-	42 (9.7)	31.1(26.8-35.4)
LT/patient-year	-	0.20	-	0.097	-

*In parenthesis are reported standard deviations or percentages as appropriate

Table 2. Italian cohort reclassification of muscle damage according to Alberta's criteria for myosteatorsis

Prior Rome classification	Reclassification according to Alberta's criteria		Total n of patients		
Myosteatorsis*					
	No myosteatorsis	myosteatorsis			
No myosteatorsis	131	0	131		
myosteatorsis	96	206	302		
total	227	206	433		
Muscle damage phenotype¶					
	No md	I-sarco	I-myo	Sarco+myo	
No md	96	0	0	0	96
I-sarco	0	35	0	0	35
I-myo	55	0	111	0	166
Sarco+myo	0	41	0	95	136
total	151	76	111	95	433

* Criteria for myosteatorsis. **Previous** publication of the Italian cohort: muscle radiodensity <41 HUs with a BMI <24.9 kg/m² and <33 HU with BMI ≥25 kg/m². New criteria proposed by Ebadi: muscle radiodensity <33 HU in males and <28 HU in females

¶ No md: no muscular damage; I-sarco: isolated sarcopenia; I-myo: isolated myosteatorsis; Sarco+myo: sarcopenia and myosteatorsis

Table 3. Adjusted sub-Hazard Ratios of myosteatosi and Sarcopenia for death by the Fine and Gray model in the Italian and Canadian cohorts according to Alberta's criteria*.

Parameter	SHR	P	95% CI
Italian cohort, full model			
Albumin	0.71	0.064	0.50-1.02
MELD	1.12	<0.0001	1.06-1.17
Ascites	1.84	0.09	0.91-3.72
Encephalopathy	2.23	0.004	1.29-3.85
Variceal bleeding	0.93	0.79	0.54-1.59
Myosteatosi	3.07	0.001	1.55-6.10
Sarcopenia	0.83	0.53	0.47-1.47
Italian cohort, final model¶			
Albumin	0.65	0.024	0.45-0.94
MELD	1.12	<0.0001	1.07-1.18
Encephalopathy	2.38	0.001	1.38-4.09
Myosteatosi	3.32	0.001	1.67-6.61
Sarcopenia	0.89	0.68	0.50-1.56
Canadian cohort, as published			
Albumin	0.55	0.007	0.36-0.55
MELD	1.04	<0.001	1.03—1.06
Ascites	1.54	0.004	1.15-2.08
Encephalopathy	1.80	<0.001	1.37-2.36
Variceal bleeding	1.57	0.02	1.09-2.25
Myosteatosi	1.47	0.001	1.17-1.84
Sarcopenia	1.55	0.001	1.24-1.94

*SHR of myosteatosi for death in the Italian cohort was adjusted by the same covariates as in Alberta's study, with each covariate coded in the same way as in Alberta's study.

¶The final model in the Italian cohort was obtained by backward elimination of non-significant covariates ($p > 0.05$) while forcing myosteatosi and sarcopenia in the model.

Table 4. Patients' reclassification of muscle damage according to the new proposed radio-intensity cutoff of 26 HU

Prior classification*	New classification by radiodensity cutoff of 26 HU		Total n of patients		
myosteatoris*					
	No myosteatoris	myosteatoris			
Italian Cohort					
No myosteatoris	131	0	131		
myosteatoris	190	112	302		
total	321	112	433		
Canadian cohort					
No myosteatoris	560	0	560		
myosteatoris	137	158	295		
total	697	158	855		
Muscle damage phenotype†					
	No md	I-sarco	I-myo	Sarco+myo	
Italian Cohort					
No md	96	0	0	0	96
I-sarco	0	35	0	0	35
I-myo	102	0	64	0	166
Sarco+myo	0	88	0	48	136
total	198	123	64	48	433
Canadian cohort					
No md	383	0	0	0	383
I-sarco	0	177	0	0	177
I-myo	75	0	72	0	147
Sarco+myo	0	62	0	86	148
total	458	239	72	86	855

*Prior classification: for the Italian cohort, muscle radiodensity <41 HUs with a BMI <24.9 kg/m² and <33 HU with BMI ≥25 kg/m²; for the Canadian cohort, muscle radiodensity <33 HU in males and <28 HU in females

Table 5. Adjusted sub-Hazard Ratios of myosteatosi and muscle damage phenotype according to the new cutoff of radiodensity (26 HU) *.

Parameter	SHR	P	95% CI
myosteatosi			
<i>Derivation Italian cohort, 425 pts</i> †			
Albumin	0.60	0.018	0.39-0.91
MELD	1.11	<0.0001	1.06-1.17
Encephalopathy	2.19	0.006	1.11-3.47
myosteatosi	1.83	0.038	1.03-3.24
Sarcopenia	0.97	0.93	0.55-1.73
<i>Validation Canadian cohort, 819 pts</i> †			
Albumin	0.89	0.219	0.75-1.07
MELD	1.04	<0.0001	1.02-1.05
Encephalopathy	2.08	<0.0001	1.60-2.71
myosteatosi¶	1.63	<0.0001	1.25-2.11
Sarcopenia	1.46	0.001	1.71-1.82
Muscle damage phenotype			
<i>Derivation Italian cohort, 426 pts</i> †			
MELD	1.13	<0.0001	1.07—1.18
Encephalopathy	2.23	<0.006	1.27-3.93
Muscle damage phenotype	1.35	0.011	1.07-1.71
<i>Derivation Italian cohort by excluding muscle damage phenotype, 426 pts</i> †			
MELD	1.13	<0.0001	1.07—1.18
Encephalopathy	2.16	<0.008	1.22-3.82
<i>Validation Canadian cohort, 827 pts</i> †			
MELD	1.04	<0.0001	1.02-1.05
Encephalopathy	2.07	<0.0001	1.59-2.68
Muscle damage phenotype	1.32	<0.0001	1.20-1.47
<i>Validation Canadian cohort excluding muscle damage phenotype, 827 pts</i> †			
MELD	1.04	<0.0001	1.02-1.05
Encephalopathy	1.93	<0.0001	1.49-2.50

*Significant variables from Fine and Gray models, including age, sex, etiology, albumin, MELD, ascites, sodium, bleeding, diabetes, BMI, myosteatosi according to the new cutoff, and sarcopenia.

† The number of patients included in the model was slightly lower than the whole population because of missing values for some of the included covariates.

¶ When removing albumin from this model, the SHR of myosteatosi changed only at the third decimal, including the confidence interval.

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Figure 1.

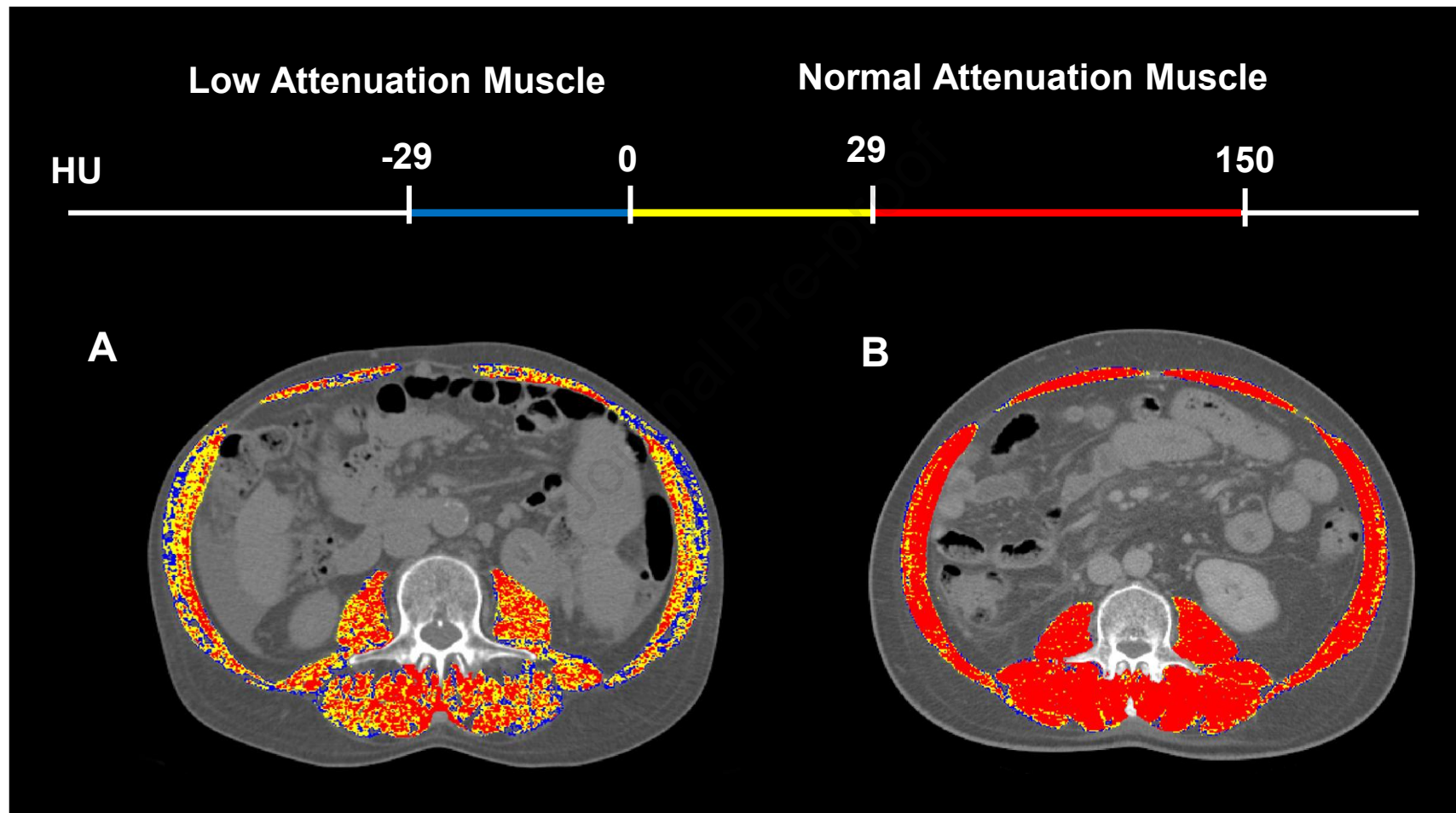


Figure 2.

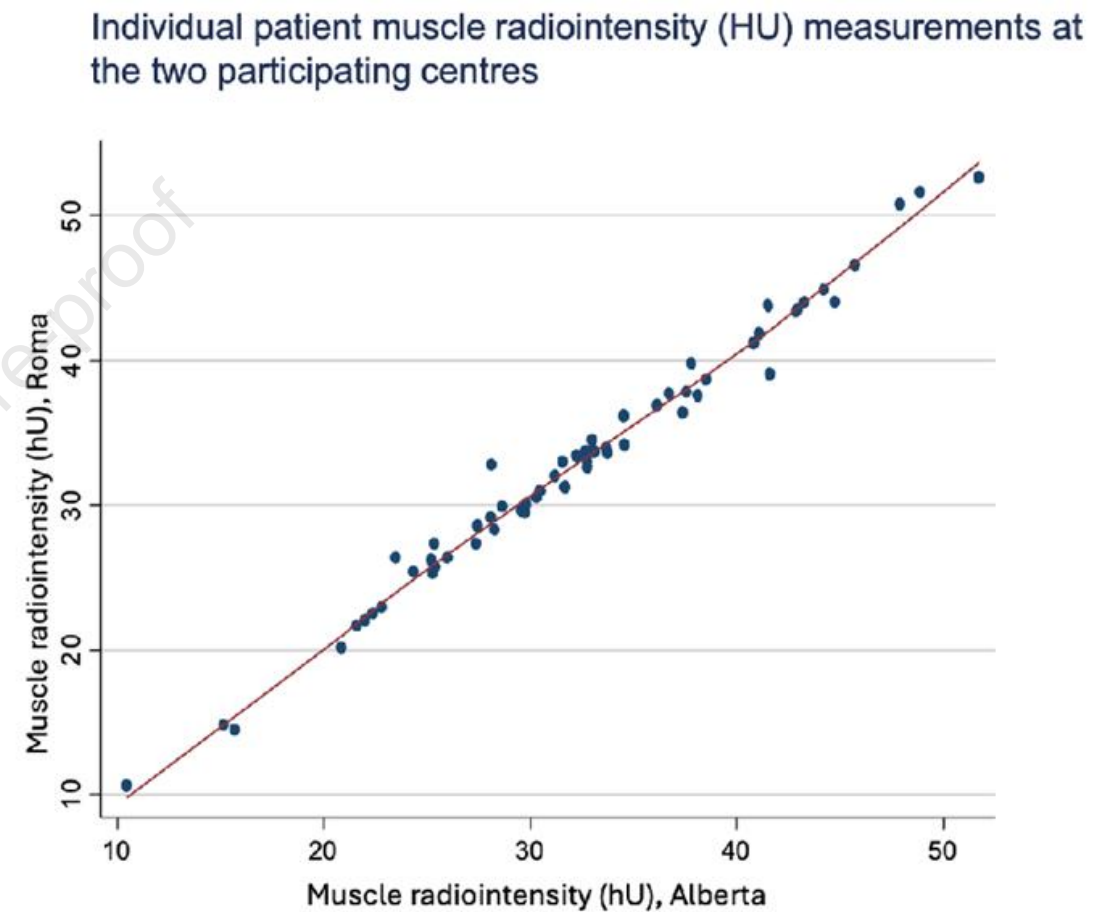
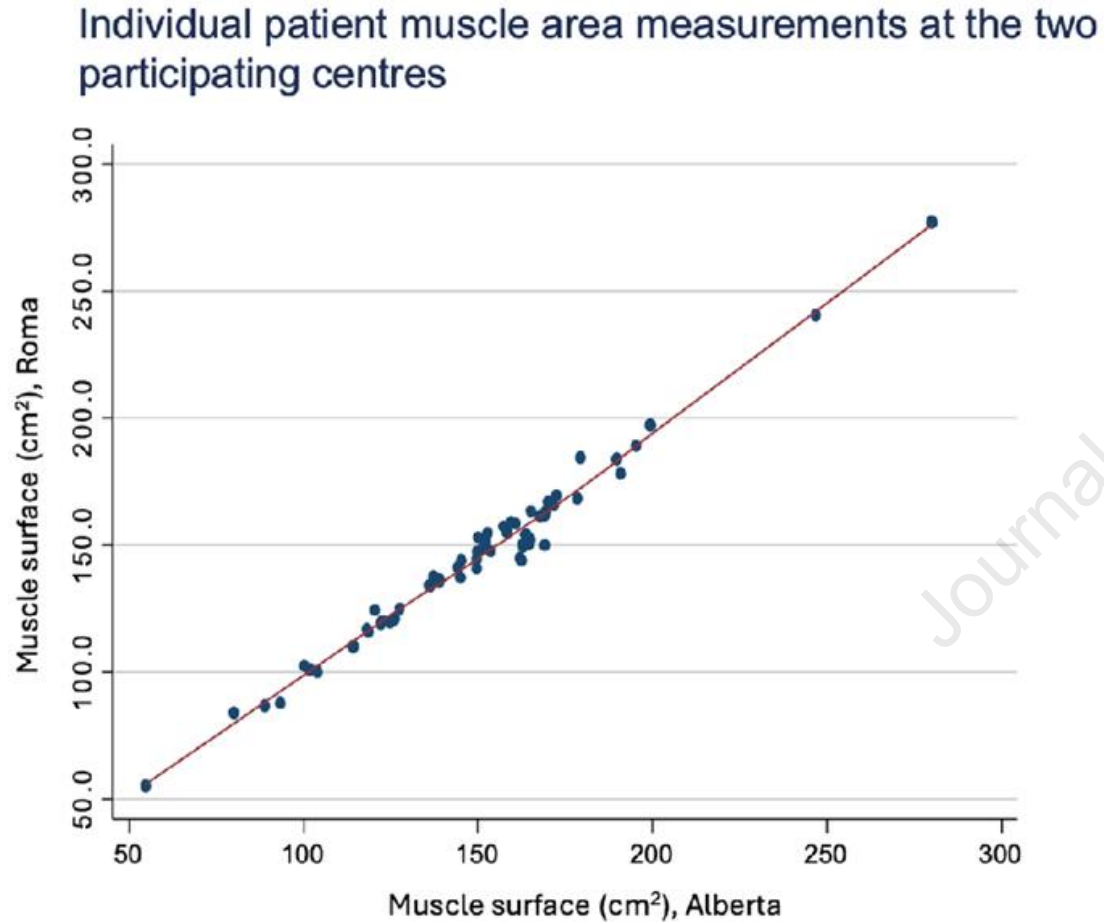
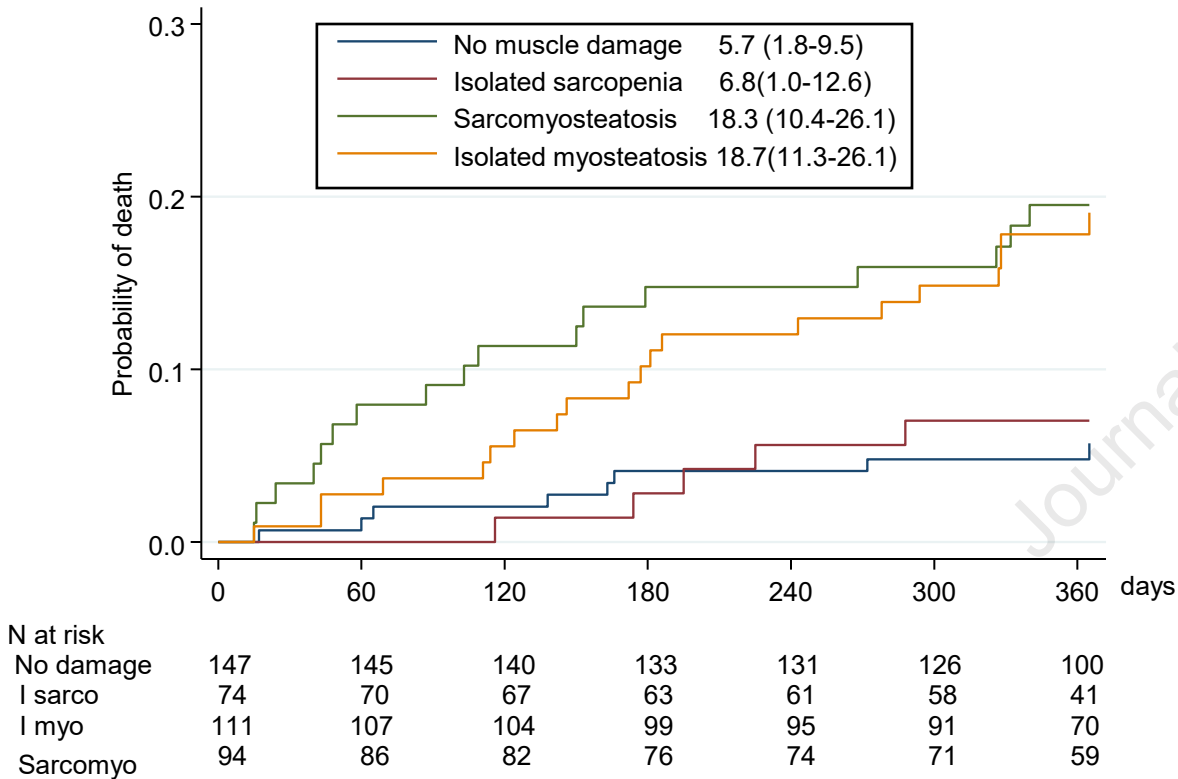


Figure 3.

1-year CIF of death in patients in the Italian cohort ty according to Ebadi's criteria for myosteatosi



1-year CIF of death in patients in the Italian cohort ty according to the Italian previously published criteria for myosteatosi

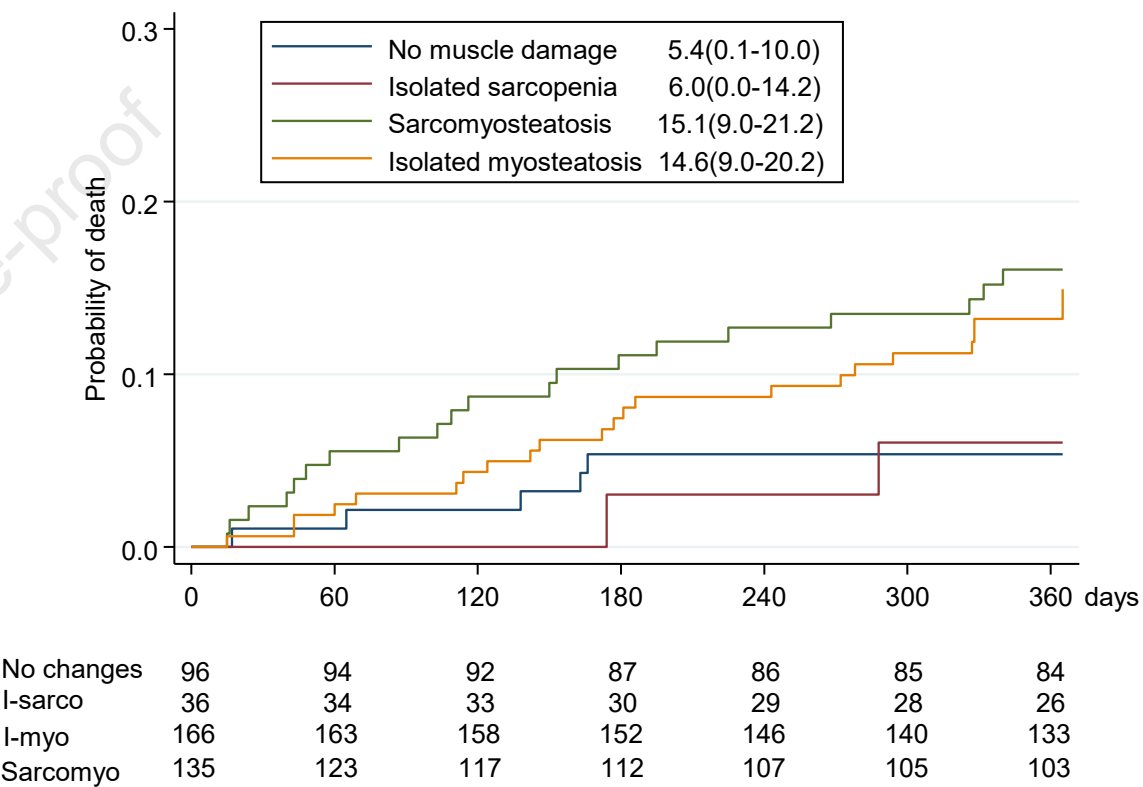


Figure 4.

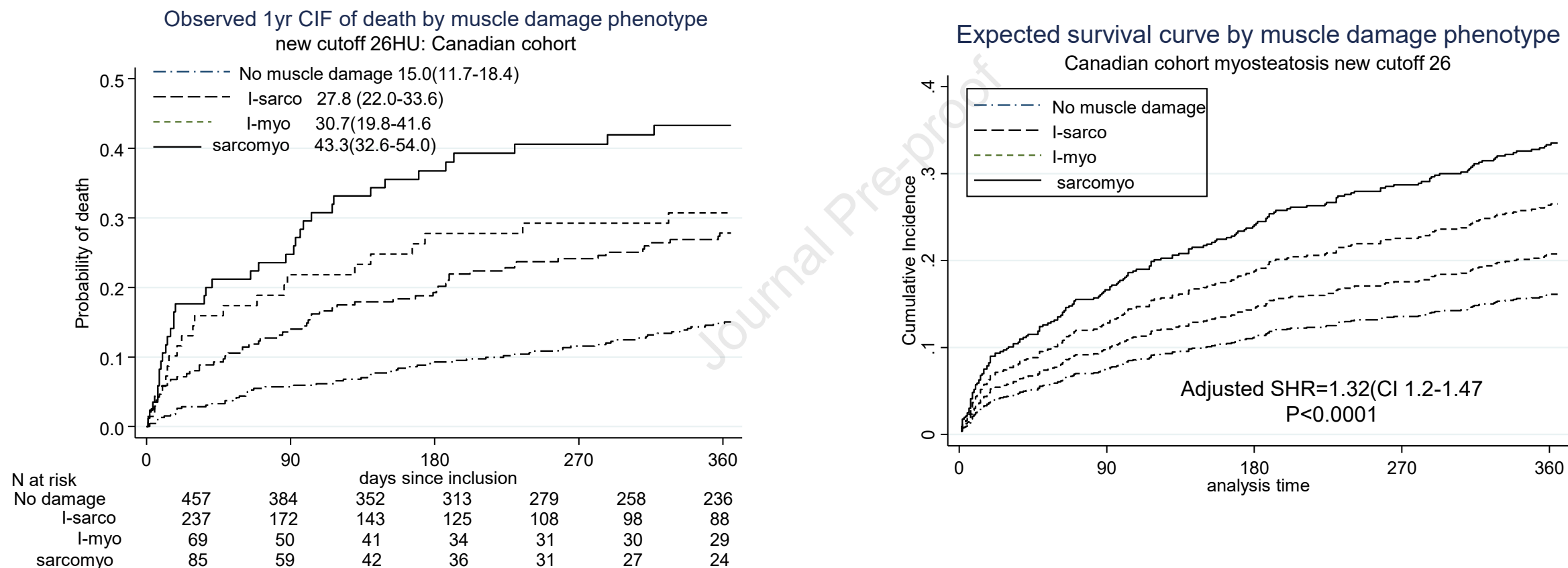
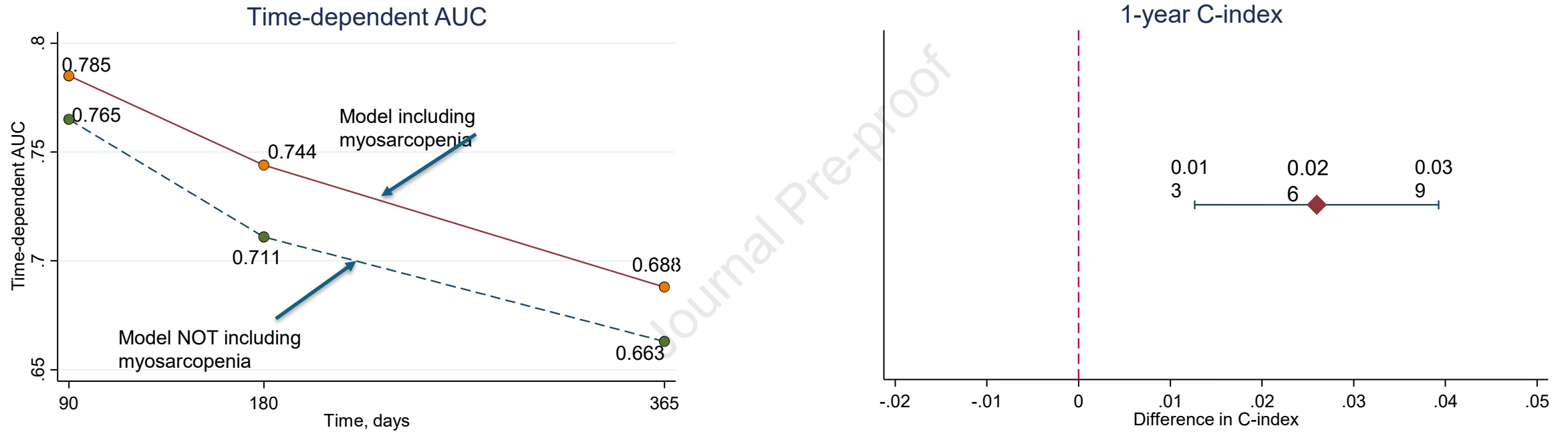
**Figure 4.** Observed (right panel) and expected CIF of death (left panel), according to the new muscle damage phenotype classification in the Canadian cohort

Figure 5.



Highlights

- Interobserver error in the assessment of myosteatosis in advanced liver disease is unknown, nor has a specific cutoff been agreed upon
- We found that muscle attenuation assessment, as a surrogate for myosteatosis, is highly reproducible across independent centres.
- A cut-off of ≤ 26 HU Hounsfield units, independent of sex and BMI, allows detecting patients with similar mortality risk across independent centres.
- If further prospectively validated, this cut-off would allow reproducible patient risk-classification across cohorts with different baseline risk.