BMJ Open

Study protocol to redefine muscle attenuation cut-offs for better prediction of mortality in patients with cirrhosis: a comprehensive post hoc validation study – a study protocol

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To cite: Di Cola S, D'Amico G, Motamedrad M, *et al*. Study protocol to redefine muscle attenuation cut-offs for better prediction of mortality in patients with cirrhosis: a comprehensive post hoc validation study – a study protocol. *BMJ Open* 2025;**15**:e094252. doi:10.1136/bmjopen-2024-094252

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2024-094252).

Received 26 September 2024
Accepted 05 March 2025

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ABSTRACT

Introduction Myosteatosis, characterised by altered muscle composition detectable by muscle radiodensity attenuation on CT scans, has been associated with increased mortality in patients with cirrhosis. However, standard attenuation cut-offs, derived primarily from oncology populations, may not be appropriate for patients with cirrhosis. This study protocol aims to address this diagnostic gap by validating the Ebadi cut-offs, which are based on a retrospective cohort and have not been extensively validated in a cirrhotic population. The aim of the study is to refine these cut-offs for more accurate prediction of mortality in patients with cirrhosis using two independent patient cohorts (retrospective and prospective).

Methods and analysis This post hoc validation study analyses muscle weakness cut-offs in patients with cirrhosis using data from two independent cohorts. A total of 1537 patients will be analysed. The study will assess interobserver variability to ensure robust results by analysing random samples of 60 patients from the two cohorts. Statistical methods will be used to determine the accuracy and relevance of current cut-offs in predicting patient mortality. The analysis will also examine the relationship between muscle wasting and clinical outcomes in cirrhosis and the relationship with muscle mass loss.

Ethics and dissemination Ethical approval for this study has been obtained from the relevant institutional review boards. The results will be disseminated through presentations at scientific conferences and publication in peer-reviewed journals. The results of the study are expected to contribute to improved diagnostic criteria for myosteatosis in cirrhosis, providing clinicians with more tailored and accurate tools for cirrhosis prognosis.

Trial registration number NCT06593015.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study will be a comprehensive analysis of individual data of a large cohort of patients with cirrhosis.
⇒ Utilisation of two independent cohorts enhances the generalisability of the study findings, offering robust external validation of the muscle attenuation cut-offs in patients with cirrhosis.
⇒ In-depth analysis of interobserver variability helps ensure the reliability and consistency of the radiological measurements.
⇒ The post hoc nature of the validation could introduce biases inherent in retrospective analyses, potentially compromising the ability of the trial to establish causality and obtain all necessary data for all patients.

BACKGROUND

Myosteatosis is defined as a pathological infiltration of fat into skeletal muscle mass, resulting in reduced muscle quality.[1] This condition is more common in the elderly and correlates with visceral fat and higher body mass index (BMI). However, chronic diseases can lead to intramuscular fat accumulation as a result of a chronic inflammatory state.[2]

Myosteatosis is the predominant muscle alteration in cirrhosis, with a prevalence of 20–80%, depending on the method of diagnosis.[3] The presence of myosteatosis in cirrhosis is associated with the risk of overt hepatic encephalopathy, prolonged hospitalisation, post-transplant complications and poor survival.[4 5]

Intramuscular fat accumulation is usually diagnosed by muscle radiodensity attenuation assessed on CT scan at the third lumbar vertebra (L3) level in Hounsfield Units (HU).[1] The normal muscle radiointensity ranges from 29 to 150 HU. As a result of muscle fat infiltration, mean muscle radiodensity decreases (muscle attenuation). The standard cut-offs of muscle attenuation to detect myosteatosis derive from a study by Martin *et al* in an oncology population[6] and vary with BMI: <33 HU in patients with

a BMI≥25 kg/m² and<41 HU in those with a BMI<25 kg/m². Moreover, the composition and distribution of fat and muscle are different in men and women, so that sex-specific cut-offs are probably needed to better identify pathological muscle wasting.

In cirrhosis, fluid accumulation (ascites and peripheral oedema) may impair the assessment of both BMI and muscle attenuation due to the low radiodensity of water relative to muscle mass. This suggests that specific attenuation cut-offs would be appropriate to detect myosteatosis in cirrhosis, at least in patients with water retention.[3]

In this context, Ebadi *et al* have suggested that in patients with cirrhosis, muscle radiodensity below 33 HU in men and 28 in women indicates myosteatosis and is associated with a higher risk of mortality, independent of BMI.[7] However, these cut-offs were obtained from a retrospective cohort and have never been validated by an external study. Moreover, the interobserver variability in assessing the presence of myosteatosis in cirrhosis has never been investigated.

Therefore, the present study aims to validate the Ebadi cut-offs in patients without and, respectively, fluid retention in patients with cirrhosis and to investigate the interobserver variability in the assessment of myosteatosis.

Study design

The study protocol was registered on ClinicalTrials.gov and is available for consultation from this link https://clinicaltrials.gov/study/NCT06593015

The study will be based on a retrospective individual patient data (IPD) analysis of two cohorts previously published and recruited in two independent centres (University of Rome[5] and University of Alberta).[7] Of the two cohorts to be joined in this IPD analysis, those included in the Ebadi study were recruited retrospectively, while the Di Cola study included a prospective cohort. The two studies will be referred to as the 'Canadian' and 'Italian' cohorts throughout this protocol.

The interobserver variability on the diagnosis of myosteatosis, according to the proposed cut-offs, will be assessed by the K-index for two raters over 60 patients, blindly assessed by two expert observers from the two participating centres. Similarly, the interobserver variability of the diagnosis of sarcopenia will be assessed in the same group of patients. We will calculate the intersoftware and interobserver and intraobserver agreement using intraclass correlation coefficients with 95% CI using a two-way mixed single measures model with absolute agreement.

Validation of the Ebadi cut-offs for myosteatosis will be assessed by comparing the sub-HRs (SHRs) for the survival of patients grouped according to these cut-offs in the two studies. SHRs will be computed by the Fine and Grey proportional hazard model with liver transplantation (LT) as the competing event.

Data collection from both cohorts started in January 2025 and is scheduled to end in March 2025, while statistical analysis will be completed by June 2025.

Inclusion criteria in the two studies

- For the Canadian cohort: adult patients with cirrhosis who had a CT image obtained as part of the LT evaluation.
- For the Italian cohort: all patients with cirrhosis aged 40–75 years who underwent an abdominal CT scan for any clinical indication. The cut-off age was an arbitrary decision to reduce the confounding effect of age-related muscle changes.

Exclusion criteria were:

- For the Canadian cohort: absence of cirrhosis, multiorgan transplantation or retransplantation.
- For the Italian cohort: (1) patient on LT waiting lists, (2) hepatocellular carcinoma, (3) history of LT, (4) concomitant neuromuscular disease, (5) current malignancy other than non-melanocytic skin cancer, (6) history of serious extrahepatic disease, (7) HIV infection.

Patients were enrolled at the time of abdominal CT scan in both cohorts.

In the Canadian cohort, 1104 patients were evaluated for LT between January 2000 and August 2021 in the University of Alberta Hospital (Canada), of whom 241 were excluded for not meeting the inclusion and exclusion criteria, so 863 patients were considered for the analysis.

In the Italian cohort, 447 patients were prospectively enrolled from 26 Italian centres, 14 of whom were excluded due to incomplete data, leaving 433 patients for the analysis.

Ethics and dissemination

Data were collected from two originator studies. For the Italian cohort, the local ethics committee of the coordinating centre approved the study protocol and data collection (EC n° 94/19, 30/01/19) and each collaborating centre provided its own ethics committee approval. All patients gave informed consent to participate in the study, and data were anonymised. For the Canadian cohort, the University of Alberta Institutional Review Board reviewed and approved the study (Pro00066572).

Patient and public involvement statement

None.

Data collection

In online supplemental table 1, the types of data collected from individual participants at the time of enrolment in each of the two cohorts are reported. Data from both cohorts will be reported in a dedicated database. In order to ensure the homogeneity and accuracy of the data to be analysed, a detailed and standardised legend of variables and definitions has been created, as shown in online supplemental table 2.

The median age was 56±8 years and 57±9 years in the Canadian and Italian cohorts respectively. Male patients were 63% in the Canadian cohort and 71% in the Italian cohort. The predominant aetiology of cirrhosis in the

Canadian cohort was hepatitis C (39%), followed by alcohol (25%) and Non-alcoholic steatohepatitis (NASH, 20%), while in the Italian cohort, alcohol was reported as the main aetiology (39.9%), followed by hepatitis C virus (15.5%) and NASH (15%). Baseline mean±SD Model of End Stage Liver Disease (MELD) score was 15±8 in the Canadian and 14.3±5 in the Italian cohorts, respectively. The Canadian study included 860 patients with a mean follow-up of 24±35 months for a total of 1770 patient-years. 369 patients died, 349 received LT and 142 were censored at the last follow-up. The 349 patients who received LT were transplanted after a mean of 12.6±22.1 months. The overall incidence of death was 0.2 deaths/patient year. In the Italian study, 433 patients were followed for a mean of 349±89 days, resulting in a total of 414 patient-years and an incidence rate of death of 0.13/patient-year. The difference is probably related to a different distribution of prognostic indicators at enrolment, which we will be able to assess based on IPD analysis.

Baseline assessment of muscle damage

Radiological assessment of muscle mass and quality was performed in both Canadian and Italian cohorts using CT scans and sliceOmatic software (V.4.2; Tomovision, Montreal, QC, Canada). In the Canadian cohort, abdominal CT scans were evaluated at the third lumbar vertebra. In the Italian cohort, muscle area was assessed by CT at the third or fourth lumbar vertebra. Therefore, in case appreciable interobserver variability is found, the level for assessing the muscle mass will be unified and the interobserver variability study will be repeated.

In both cohorts, the HU thresholds for assessing skeletal muscle mass ranged from −29 to +150 HU. The muscle area was adjusted for height to obtain a ratio (cm^2/m^2), referred to as the L3 or L3–L4 skeletal muscle index (SMI). In both Italian and Canadian cohorts, patients were diagnosed with sarcopenia based on the validated SMI cut-off values: less than 50 cm^2/m^2 for men and less than 39 cm^2/m^2 for women.[8]

Mean muscle attenuation in L3 or L3/L4 muscle mass assessed on CT scan was used to diagnose myosteatosis.

In the Italian cohort of patients, myosteatosis was defined as radiodensity <41 HUs with a BMI below 24.9 kg/m^2 and <33 HUs with a BMI ≥25 kg/m^2.[6]

Ebadi M *et al* have identified new sex-specific cut-offs for the diagnosis of myosteatosis using continuous muscle attenuation values in a competitive risk analysis; patients with muscle attenuation less than 33 HU in males and 28 HU in females had a significantly higher risk of mortality during follow-up.

In both cohorts, patients were classified based on the presence and type of muscle changes, as follows:

- No myosteatosis—no sarcopenia (no muscle changes): 45% in the Canadian study, 22.2% in the Italian study.
- Myosteatosis—no sarcopenia (isolated myosteatosis): 17% in the Canadian study, 38% in the Italian study.
- No myosteatosis—sarcopenia (isolated sarcopenia): 22% in the Canadian study, 8.3% in the Italian study.
- Myosteatosis—sarcopenia (combined sarcopenia and myosteatosis): 17% in the Canadian study, 31.2% in the Italian study.

Since myosteatosis was defined differently in the two studies, patients in the Di Cola study will be reclassified according to the Ebadi criteria for myosteatosis. The whole population from the two studies will then be classified according to the four prognostic groups above reported.

Baseline clinical characterisation, follow-up and events

Baseline clinical characteristics were recorded at the time of the radiological assessment in both cohorts and included: demographics, laboratory parameters, aetiology of liver disease, decompensation and type of decompensating event. If not available, information on prior decompensation and type of prior decompensation will be retrieved in available databases or from clinical records. For the scope of the present study, decompensation will be defined by the presence or prior occurrence of any of ascites, portal hypertensive bleeding, encephalopathy or jaundice (bilirubin >3 mg/dL). In the Canadian cohort, patients were followed for a mean time of 24±35 months. In the Italian cohort, patients were followed for 12 months after enrolment. In both cohorts, patients were visited approximately at 6-month intervals or whenever clinically needed, and major clinical events were recorded. The 12-months follow-up for each patient will be considered for the present study.

Primary and secondary outcomes

The primary outcome for the present study will be overall mortality. LT will be considered as a competing event in any analysis of the clinical impact of muscle damage, according to the above definition and classification.

Secondary outcomes will be hospitalisation for liver-related reasons and further decompensation. Hospitalisation for liver-related reasons will be defined as any hospitalisation for any complication of cirrhosis, including day hospital admissions for paracentesis, endoscopic treatments for oesophageal varices and transjugular intrahepatic portosystemic shunts.

Further decompensation will be defined according to the Baveno VII criteria.[9]

Assessment of interobserver variability in the diagnosis of muscle damage

This will be based on a random sample of 60 patients from the two cohorts: 30 per each of the two cohorts included in this study. The 30 patients from the Rome study cohort will be blindly assessed by an expert from the Alberta study. Correspondingly, 30 patients randomly selected by the Alberta study cohort will be blindly assessed by one expert in Rome. The randomisation will be stratified by the previous adjudication of myosteatosis and sarcopenia in each of the two study cohorts to include approximately similar numbers of patients with/without sarcopenia and with/without myosteatosis. The sample size will be

further expanded in case of appreciable difference in the subgroups composition with regard to presence/absence of sarcopenia and, respectively, myosteatosis, to achieve a similar number of patients across subgroups.

Validation of the Ebadi cut-offs for myosteatosis

Patients in the Italian cohort will be classified with myosteatosis according to the Ebadi cut-offs and the above-reported classification. The clinical impact of myosteatosis and of this classification on mortality will then be assessed in this cohort by the Fine and Gray proportional hazards model for competing risks with LT considered as a competing event. The SHR of myosteatosis for mortality in the Italian cohort will be compared with that reported by Ebadi[7] after adjustment for the same covariates as in the Ebadi study. The radiointensity attenuation cut-offs for detecting myosteatosis and the proposed classification will be considered satisfactorily valid if the differences between the SHRs in the Ebadi study and the Italian cohort are not statistically significant with p>0.10.

Statistical analysis

Analyses will be conducted according to the World Medical Association (WMA) Declaration of Helsinki and according to the intention-to-treat principle will include all the patients included in the two original studies.[5 7]

Baseline patient characteristics will be assessed as proportions with 95% CI, means with SD or medians with ranges as appropriate. Differences between proportions will be assessed by the χ^2 test and between means by the student's t-test where appropriate.

Interobserver variability for the diagnosis of sarcopenia and, respectively, of myosteatosis will be assessed as above described, and statistical significance will be tested by the Kappa index for two raters (agreement beyond chance):[10 11] the statistical test measures the probability that the interobserver agreement beyond chance is significantly different from zero.

Survival will be assessed by the Kaplan-Meier method[12] and differences between patient subgroups by the log-rank test. The cumulative incidence function (CIF) of major clinical events will be assessed by a competing risks model,[13 14] where LT will be the competing event; death will also be a competing event when assessing the incidence of hospitalisation or further decompensation. Differences in CIFs of relevant events between groups will be assessed by Gray's test using Lambert's procedure for the Stata software.[15]

Validation of the Ebadi cut-offs of muscle radiointensity attenuation will be based on the comparison of the SHRs for death in patient subgroups defined by the relevant cut-offs, observed in the Ebadi and in the Di Cola series, respectively. SHRs of the myosteatosis cut-offs for mortality will be derived by multivariable proportional hazards model for competing risks[16] and adjusted for the same prognostic variables used in the Ebadi study.[7]

Ebadi cut-offs will be considered satisfactorily valid if the SBHs for mortality do not significantly differ in the two studies with p>0.10 (or 90% CI overlap).

The cumulative incidences of any hospitalisation for any complication of cirrhosis and of further decompensation will be assessed also by competing risks analyses with death and LT as competing events.

Sensitivity validation analyses will be performed in compensated/decompensated patients and according to aetiology (MAFLD vs viral/other).

Prognostic analyses for death, hospitalisation and further decompensation are also performed using the Fine and Gray model including the following variables (list here the predefined candidate prognostic variables including time of CT scan acquisition). For each analysis, the number of included variables will be kept lower than 1 per 10 observed outcome events. The analyses will be performed by non-automated backward procedure, with variables to be kept in the model chosen on the basis of their known prognostic weight, clinical judgment and statistical significance at p<0.05.

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Contributors MM: study concept and protocol, drafting and revision of the manuscript for important intellectual content. SDC: study protocol, analysis plan, drafting and revision of the manuscript. GDA: study protocol, analysis plan, results interpretation, drafting and revision of the manuscript. AM-L: drafting and revision of the manuscript for important intellectual content. MM: drafting and revision of the manuscript for important intellectual content. MM is the guarantor of the contributorship.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Provenance and peer review Not commissioned; externally peer reviewed.

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