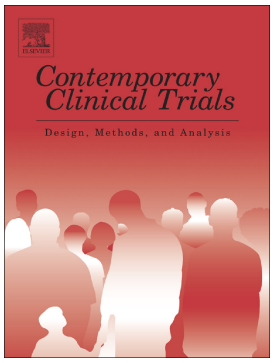
Liver investigation: Testing marker utility in steatohepatitis (LITMUS): Assessment & validation of imaging modality performance across the NAFLD spectrum in a prospectively recruited cohort study (the LITMUS imaging study): Study protocol



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Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS): Assessment & Validation of Imaging Modality Performance across the NAFLD Spectrum in a Prospectively Recruited Cohort Study (The LITMUS Imaging Study): Study Protocol

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Visualisation: MP
Writing, original draft preparation: MP
Writing, review and editing: all authors

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MP receives support from the Oxford NIHR Biomedical Research Centre. QMA receives support from the Newcastle NIHR Biomedical Research Centre and is an NIHR Senior Investigator.
Andrew Blamire supervises MRI in Newcastle, UK.

Disclaimer

This communication reflects the view of the author(s) and neither IMI nor the European Union or EFPIA are liable for any use that may be made of the information contained herein.

Conflict of Interest

FEM, SA, KW, JC, MA, EMG, SF, MRG, JC, IFL, RA, RSG, JMP, JB, VR, MW, SP, MA, RF, LM, ET, ME, PL, ATH, GP, HYJ, KP and PMB declare no conflicts of interest.

MP is a shareholder in Perspectum Ltd.

EMT is a shareholder in Perspectum Ltd.

SA is currently employed by Boehringer Ingelheim (but was not during his participation in the project)

GPA has served as a consultant and an advisory board member for Pfizer Inc, Inventiva Pharma, GlaxoSmithKline and KaNDy Therapeutics; he has been a consultant to BerGenBio ASA, Median Technologies, FRACTYL, Amryt Pharmaceuticals and AstraZeneca; and has given presentations on behalf of Roche Diagnostics and Medscape all through the University of Nottingham contract.

CA declares the following potential conflicts of interest: Hologic: Support of study and expert; Guerbet: Member of board, PI of study; Siemens: Expert.

EB has served as a consultant or advisory board member for Boehringer Ingelheim, Gilead Sciences, Intercept, Merck, Novo Nordisk, Pfizer, ProSciento; and a speaker for Gilead Sciences, Intercept, Merck, Novo Nordisk, Pfizer. She has also received a research grant from Gilead Sciences for fatty liver research.

AG served as a speaker and consultant for AbbVie, Alexion, AstraZeneca, Bayer, BMS, CSL Behring, Eisai, Falk, Gilead, Heel, Intercept, Ipsen, Merz, MSD, Novartis, Pfizer, Roche, Sanofi-Aventis, Sequana; received research funding from Intercept, Falk, Novartis.

JMS reports consultancy for BMS, Boehringer Ingelheim, Echosens, Genfit, Gilead Sciences, Intercept Pharmaceuticals, Madrigal, Novartis, Pfizer, Roche, Sanofi; received research funding from Gilead Sciences and was on the speaker's bureau for Falk Foundation MSD Sharp & Dohme GmbH.

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RLE and the Mayo Clinic have intellectual property rights and a financial interest in magnetic resonance elastography technology.

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TT was employed by Pfizer at the time of her involvement with the project.

QMA is coordinator of the EU IMI-2 LITMUS consortium, which is funded by the EU Horizon 2020 programme and EFPIA. This multistakeholder consortium includes industry partners. QMA has received research grant funding from AstraZeneca, Boehringer Ingelheim, and Intercept Pharmaceuticals, Inc.; has served as a consultant on behalf of Newcastle University for Alimentiv, Akero, AstraZeneca, Axcella, 89bio, Boehringer Ingelheim, Bristol Myers Squibb, Galmed, Genfit, Genentech, Gilead, GSK, Hanmi, HistoIndex, Intercept Pharmaceuticals, Inc., Inventiva, Ionis, IQVIA, Janssen, Madrigal, Medpace, Merck, NGM Bio, Novartis, Novo Nordisk, PathAI, Pfizer, Poxel, Resolution Therapeutics, Roche, Ridgeline Therapeutics, RTI, Shionogi, and Terns; has served as a speaker for Fishawack, Integritas Communications, Kenes, Novo Nordisk, Madrigal, Medscape, and Springer Healthcare; and receives royalties from Elsevier Ltd.

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SAH has research grants from Akero, Altimmune, Axcella-Cirius, CiVi Biopharma, Cymabay, Galectin, Genfit, Gilead Sciences, Hepion Pharmaceuticals, Hightide Therapeutics, Intercept, Madrigal, Metacrine, NGM Bio, Northsea Therapeutics, Novartis, Novo Nordisk, Poxel, Sagimet, Viking. He has received consulting fees from Akero, Altimmune, Alentis, Arrowhead, Axcella, Echosens, Enyo, Foresite Labs, Galectin, Genfit, Gilead Sciences, Hepion, HIghtide, HistoIndex, Intercept, Kowa, Madrigal, Metacrine, NeuroBo, NGM, Northsea, Novartis, Novo Nordisk, Poxel, Perspectum, Sagimet, Terns, and Viking.

Keywords
NASH; Liver Multiscan; Iron corrected T1; T2*; Magnetic resonance elastography; Diffusion weighted imaging; T1 mapping; Proton density fat fraction; PDFF; R2*; DeMILI; Fibro-MRI; NASH-MRI; ultrasound elastography; liver stiffness; 2D shear wave elastography; 2DSWE; point shear wave elastography; pSWE; vibration controlled transient elastography; VCTE

Abstract

Non-alcoholic fatty liver disease (NAFLD) is the liver manifestation of the metabolic syndrome with global prevalence reaching epidemic levels. Despite the high disease burden in the population only a

small proportion of those with NAFLD will develop progressive liver disease, for which there is currently no approved pharmacotherapy. Identifying those who are at risk of progressive NAFLD currently requires a liver biopsy which is problematic. Firstly, liver biopsy is invasive and therefore not appropriate for use in a condition like NAFLD that affects a large proportion of the population. Secondly, biopsy is limited by sampling and observer dependent variability which can lead to misclassification of disease severity. Non-invasive biomarkers are therefore needed to replace liver biopsy in the assessment of NAFLD. Our study addresses this unmet need.

The LITMUS Imaging Study is a prospectively recruited multi-centre cohort study evaluating magnetic resonance imaging and elastography, and ultrasound elastography, against liver histology as the reference standard. Imaging biomarkers and biopsy are acquired within a 100-day window. The study employs standardised processes for imaging data collection and analysis as well as a real time central monitoring and quality control process for all the data submitted for analysis. It is anticipated that the high-quality data generated from this study will underpin changes in clinical practice for the benefit of people with NAFLD.

Study Registration: clinicaltrials.gov: NCT05179721

Background and rationale

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome, and is usually associated with obesity, type 2 diabetes mellitus (T2DM), and dyslipidaemia (1, 2). NAFLD is now the most common liver disease in Western countries, affecting up to a third of adult populations

(3, 4). The prevalence of NAFLD in people with T2DM is estimated at 43%, and at 90% in those with hyperlipidaemia or those with morbid obesity undergoing bariatric surgery (5-7). An advanced form of NAFLD, non-alcoholic steatohepatitis (NASH), is projected to be the principal aetiology for liver transplantation within the decade. Importantly, there is a growing body of clinical and epidemiological evidence suggesting that NAFLD leads to liver-related mortality as well as worsening insulin resistance and increased risk of ischaemic heart disease and stroke (1, 8). NAFLD has become one of the main concerns for practising hepato-gastroenterologists and endocrinologists due to its potential to progress to advanced liver disease and metabolic complications (1, 9).

NAFLD is broadly defined as the accumulation of lipid droplets in more than 5% of hepatocytes in the absence of excess alcohol consumption or other factors that cause secondary liver fat deposition (e.g. drugs, viruses). NAFLD is an umbrella term that encompasses a spectrum of liver pathology, characterised by isolated liver fat accumulation (simple steatosis or non-alcoholic fatty liver; NAFL) at the mild end of this spectrum. The disease progresses through varying grades of necroinflammation and hepatocyte injury (non-alcoholic steatohepatitis, NASH) and fibrosis to cirrhosis. At the most severe end of the spectrum, hepatocellular cancer and/or liver failure can develop(1).

The reliance on liver biopsy for the diagnosis and severity assessment of NAFLD presents many challenges in clinical practice and clinical trials. Liver biopsy is an invasive, resource-intensive procedure that carries a recognised risk of complications, so it cannot be applied to the whole population at risk. Furthermore, liver biopsy is limited by sampling and observer-dependent variability (10-12) which are particularly problematic in the context of clinical trials that assess response to intervention based on histological endpoints. There is therefore a need for non-invasive biomarkers that can be used instead of liver biopsy both in clinical practice and in clinical trials.

For the purpose of regulatory approvals, the intended context in which a biomarker will be used has to be clearly defined(13). Examples of contexts in which biomarkers may be utilised include use for diagnostic, prognostic, and monitoring purposes(14). While several "wet" (based on results from blood tests)(15, 16) and "dry" (based on elastography and imaging)(17) techniques have been developed, so far only one such test has received regulatory approval for use as a prognostic test(18). Limited head-to-head comparison data on biomarker performance are available, largely focussed in blood-based biomarkers (19). The need, therefore, remains for biomarkers that can be used in other contexts like screening and diagnosis as well as predicting and monitoring treatment response. Ultimately, biomarkers are needed that can replace biopsy as surrogate endpoints in NASH clinical trials.

The "Liver Investigation: Testing Marker Utility in Steatohepatitis" (LITMUS) consortium is a collaboration of academic, clinical and industry partners aiming to identify and validate biomarkers of NASH and fibrosis in people with NAFLD (https://www.imi.europa.eu/projects-results/project-factsheets/litmus). The LITMUS Imaging Study is conducted within this remit, with the specific aim of evaluating elastography and imaging biomarkers against histological assessment as reference standard.

Methods

Overview

The LITMUS Consortium is conducting two parallel studies evaluating serum-based, elastography and imaging biomarkers. The European NAFLD Registry (NCT04442334) (20) is the main study being carried out by the LITMUS Consortium. This includes a prospectively recruited cohort of participants who are having liver biopsy for NAFLD assessment as part of their routine care (The LITMUS study cohort) from whom biological samples and clinical data are collected. The LITMUS Imaging Study (NCT05479721) is running in parallel to the European NAFLD Registry and aims to evaluate a subset of participants from the LITMUS study cohort (Supplementary Figure 1). The LITMUS Imaging Study includes magnetic resonance imaging / elastography, and ultrasound elastography biomarkers.

Objectives

The primary objective is to identify non-invasive imaging modalities that accurately stage the severity of liver fibrosis in people with NAFLD, using liver histology as the reference standard.

Secondary Objectives:

- Identification of non-invasive imaging modalities that can effectively distinguish non-alcoholic steatohepatitis (NASH) from simple steatosis, using liver histology as the reference standard;
- Evaluation of imaging biomarkers for the prediction of long-term outcomes in people with NAFLD;
- Study of the natural history of NAFLD and its impact on prognosis;
- Evaluation of the reproducibility and observer-dependent variability in reporting of liver imaging biomarkers;
- Identification of physiological factors that confound the performance of imaging biomarkers for the assessment of fibrosis;

- Identification of non-invasive imaging modalities that accurately quantify liver fat and liver iron in people with NAFLD;
- Assessment of agreement between different MRI measures of liver fat.

Organisation and oversight

The LITMUS Imaging Study operates across multiple territories with an organisational and oversight structure mirroring that of the European NAFLD Registry (20) with tiered central leadership and coordination, national oversight and site delivery.

The central leadership team provides coordination of activities, including defining the master clinical study protocol. Furthermore, the central leadership team oversees a process of site qualification prior to sites submitting study data for analysis and an ongoing real-time process of quality assurance checks to ensure all submitted data are appropriate for analysis. The central leadership team includes the LITMUS Imaging Study chief investigator, project manager, data manager, representatives from the imaging analysis core labs and other LITMUS consortium partners participating in the LITMUS Imaging Study.

The conduct of the study according to these centrally defined processes is delegated to National leads and site investigators who are responsible for study sponsorship, ethical and regulatory approvals and recruitment (**Supplementary Table 1**). At sites that collect magnetic resonance data a senior MR imaging investigator oversees the site qualification process and ongoing data acquisition.

In all territories, the study is conducted according to the Declaration of Helsinki and all participants give written informed consent prior to inclusion. Each site has to gain appropriate ethical and regulatory approvals prior to recruiting study participants. Details of the associated ethical approvals for each country are detailed in **Supplementary Table 2**.

Imaging biomarkers and imaging core labs

The LITMUS Imaging study includes the following magnetic resonance imaging / elastography and ultrasound elastography biomarkers. The rationale for including these biomarkers is detailed in the supplementary methods.

Magnetic Resonance biomarkers

All MR data are collected and analysed centrally by imaging analysis core labs provided by four LITMUS partners. Each core lab is responsible site qualification, training and ongoing support at sites and analysis for the following MR modalities and biomarkers:

1. Perspectum Ltd (Oxford, UK)

Responsible for Liver*MultiScan* to compute the biomarkers of:

a. Iron corrected T1 (cT1; ms)

b. Perspectum PDFF (%)

c. T2* (ms)

2. Antaros (Mölndal, Sweden)

Responsible for MRE, DWI and T1 measured using the T1 relaxation time measured using scanner manufacturer sequences (vendor-specific T1; vT1; ms) to compute the biomarkers of:

a. MRE Liver stiffness (kPa)

b. Apparent diffusion coefficient (mm^2/s)

c. Vendor-T1 relaxation time (ms)

3. Seville imaging core lab (Seville, Spain)

Responsible for deMILI to compute the biomarkers of:

a. NASH_MRI (0-1)

b. Fibro_MRI (0-1)

4. Resoundant (Rochester, USA)

Responsible for PDFF measured using scanner manufacturer sequences (vendor-PDFF; vPDFF; %) to compute:

a. Vendor- PDFF (%)

b. R2* (ms^{-1})

Ultrasound Elastography biomarkers

Ultrasound elastography data are acquired at the recruiting sites by local investigators. The following techniques are included:

1. Liver stiffness measured using 2D shear wave elastography (2D-SWE)

2. Shear wave speed measured using point Shear Wave Elastography (pSWE)

3. Liver Stiffness by Vibration Control transient elastography (LSM-VCTE; Fibroscan, Echosens, Paris, France). The LSM-VCTE data are captured in the European NAFLD Registry study and are made available with integration with other data from the LITMUS Imaging Study and downstream analysis.

Inclusion / Exclusion criteria

The study population comprises adults (aged ≥18 years <80) who undergo liver biopsy for the evaluation of NAFLD. Study participants are recruited from hepatology clinics and/or bariatric surgery units. All participants in the LITMUS Imaging Study must also participate in the European NAFLD Registry (20) and thus fulfil the inclusion/exclusion criteria of that study.

The specific inclusion and exclusion criteria for the LITMUS Imaging Study are:

Inclusion criteria:

1. Recruited to the European NAFLD Registry

2. Liver biopsy for the assessment of NAFLD done within (+/-) 100 days of study assessments

3. Participant is willing and able to give informed consent for participation in the study.

Exclusion criteria

1. Not a speaker of the native language of the territory where the study is being conducted and unable to access an interpreter. Due to the nature of the study, understanding the native language or access to a relevant interpreter is a necessary criterion for participant's safety regarding MR scanning.

2. People judged by the investigator to be unsuitable for inclusion in the study (e.g. where the investigator feels that the participant will not be able to comply with the study procedures)

3. Any contraindication to MRI (e.g. ferrous metal implants/fragments, implantable cardiac defibrillator or permanent pacemaker, metal clips following neurosurgery, pregnancy, other condition that would make MR scanning unsafe in the opinion of the scanner operator).

Study Procedures

Screening, recruitment, and informed consent

The site principal investigator and their team identify eligible people with NAFLD who are then invited to participate in the study. Recruitment can take place before or after clinically indicated liver biopsy for the evaluation of suspected NAFLD. Participants must also be recruited by the European NAFLD Registry. All participants provide written informed consent.

Study visits

The procedures in the LITMUS Imaging Study take place outside the participants' routine clinical care and in general a dedicated research visit is required. Where possible, these are scheduled at times when the participant is attending the hospital for other clinical purposes.

Baseline visit 1

At baseline visit 1, participants undergo a magnetic resonance (MR) scan and / or an ultrasound elastography scan depending on the site capabilities (**Supplementary Table 3**). The magnetic resonance scans include Liver*MultiScan* (Perspectum Ltd; LMS), MRI for the detection of metabolic liver injury (deMILI), liver diffusion-weighted imaging (DWI), liver proton density fat fraction acquired using the scanner manufacturer sequences (vendor-PDFF; vPDFF), T1 acquired using the scanner manufacturer sequences (vendor-T1; vT1) and magnetic resonance elastography (MRE). Ultrasound-based elastography techniques include 2-dimensional shear wave elastography (2D-SWE) and point shear wave elastography (pSWE). The MR assessments take 30-45 minutes, depending on availability of different modalities at the study site and are performed either on a 1.5T or 3.0T MR scanner. The US elastography assessment typically takes place at the time of clinically indicated ultrasound-guided liver biopsy. This adds an additional 5-10 minutes to the scan required as part of the clinical care. If the ultrasound has not been performed as part of the clinical care biopsy procedure, a research ultrasound elastography scan is performed (15-20 minutes). Participants attend the baseline visit having fasted for at least 4 hours. More details relating to the MR and US procedures are included in the supplementary methods.

Baseline visit 2

To assess the reproducibility of imaging biomarkers, a subset of 20 participants in the LITMUS Imaging Study will be assessed with a repeat of the baseline study procedures within 30 days of visit 1. The study procedures at baseline visit 2 are identical to the procedures at baseline visit 1.

Follow-up visit – 6 to 24months after baseline

All participants are followed up in the LITMUS Imaging Study with another assessment after 6 to 24 months. The imaging assessment at this visit are identical to the assessment at baseline 1.

Imaging data management

Imaging data acquisition

MR data are acquired according to the LITMUS Imaging protocol. Table 1 includes an overview of the MR acquisition protocol with further details in the appendix. Ultrasound elastography data are acquired according to the manufacturer recommendations.

Imaging data flow

After acquisition, MR data is labelled with the same unique study identifier that has already been assigned to the subject in the European NAFLD Registry, so that imaging and clinical data can be combined correctly at the statistical analysis stage. If an alternative study identifier is used for the LITMUS Imaging Study then this must be indicated. Data labelled with the unique study identifier are then uploaded to a secure online portal compliant with ISO 270001, and 21CFR11 provided by Perspectum Ltd (Oxford. UK). The portal allows customisable access permissions and allows data to be passed to the relevant core labs for analysis (LiMS to Perspectum; MRE, DWI and vT1 to Antaros; deMILI to Seville; vPDFF to Resoundant). The core labs provide their analysis results to the European NAFLD Registry for integration with the other data collected there. All the MR data undergo clinical reporting for the presence of any incidental findings. If such findings are present they are communicated to the clinical site for further action. Ultrasound elastography data are acquired and analysed at the site with the results of this analysis entered directly into the European NAFLD Registry. **Figure 1** illustrates the data flow in the study.

Quality assurance processes

MRI site qualification

Before collecting MR data for the study, all staff at the site imaging centre receive training on the LITMUS imaging scanning protocol and procedures. The site then submits pilot data which are checked by the relevant imaging core labs. Once the core labs are satisfied that the site is technically competent to perform the MR scanning according to the LITMUS MRI protocol, they provide technical approval.

The central study coordinating team then ensures that the required ethical approvals are in place for the site to conduct the study. The final site qualification is documented and communicated to the site, after which the site can start collecting MR data for the study.

Central monitoring

Central monitoring is conducted by the LITMUS Imaging Study coordinating team at Oxford along with the four MR core labs. The central imaging data management team checks that the unique study identifiers entered in the imaging study portal correspond to a unique study identifier in the European NAFLD Registry. Once this is established each core lab checks that their data are complete, they do not include any personal identifiable information and are of sufficient technical quality to be analysed. Cases that do not meet these quality standards are rejected (and deleted if containing personal identifying information) from the portal and the site is asked to re-upload the data after resolving the issues identified. The central data management team and core labs meet fortnightly to discuss quality control issues for scans uploaded in the previous 2 weeks. If persistent problems are identified with particular sites, core labs provide additional support and training to resolve these.

Blinding

Central to the LITMUS effort is the robust evaluation of all biomarkers. To achieve this, data are centralised in the European NAFLD Registry. Data flow into the Registry and access to the whole dataset is available only to the team performing the statistical analysis. Data do not flow out of the European NAFLD Registry to other LITMUS consortium partners. Specifically, for the LITMUS Imaging Study, clinical phenotype data including liver histology parameters and clinical outcomes are collected without knowledge of the imaging biomarker results. Likewise, central imaging biomarker analysis is performed without knowledge of clinical phenotype and outcome data.

MR data analysis

Perspectum

The LMS MOLLI, LMS IDEAL and LMS T2* are analysed using LiverMultiScan, a semi-automated post-processing tool. During image analysis, for all slices acquired, iron-corrected T1 (cT1) and PDFF maps of the liver are delineated into whole liver segmentation maps using a semi-automatic method (Figure 2). For T2*, three 15-mm diameter circular regions of interest (ROI) are placed on the transverse LMS T2* maps for each slice, covering a representative sample of the liver, to calculate average T2* values for T1-correction. Non-parenchyma structures such as bile ducts and large blood vessels as well as image artifacts are excluded from image analysis.

Antaros

Magnetic Resonance Elastography

The liver stiffness is measured as the mean shear stiffness within a volume of interest (VOI) according to the Quantitative Imaging Biomarkers Alliance (QIBA) criteria (21). Using the MRE magnitude images, the initial VOI is drawn encompassing all visible liver parenchyma while staying approximately 1 cm from the edge of the liver. Non-parenchyma structures such as bile ducts and large blood vessels as well as image artifacts are excluded from the VOI. At each slice position, magnitude images from the different phase offsets are compared and areas with visible motion between phase offsets are excluded from the VOI. The VOI is then transferred to the elastogram with the 95% confidence checkerboard overlay where areas outside the 95% confidence are excluded from the VOI. The remaining VOI is used to calculate the mean shear stiffness of the liver (Figure 3).

Diffusion-Weighted Imaging MRI

The liver Apparent Diffusion Coefficient (ADC) is measured as the median ADC within a volume of interest (VOI). Using the DW-MRI magnitude images, the initial VOI is drawn encompassing all visible liver parenchyma. Non-parenchyma structures such as bile ducts and large blood vessels as well as image artifacts and areas with inadequate Signal to Noise Ratio (SNR) are excluded from the VOI. At

each slice position, magnitude images with varying diffusion-weighting (b-values) are compared and areas with visible motion between b-value images are excluded from the VOI. The remaining VOI is transferred to the voxel wise calculated ADC map and used to measure the median ADC within the liver (Figure 3).

Vendor T1

Vendor T1 is measured in a similar fashion to the ADC measurement. Here the VOI is transferred the MRI scanner vendors' voxel wise calculated T1 maps and used to measure the median T1 within the liver parenchyma (Figure 3).

Seville

In a preliminary quality control step, the images are checked to verify that the three required MR sequences are present and performed correctly in the axial plane. In a second quality control step the images are reviewed to ensure sufficient quality and that the entire liver was imaged. Six slices from each of the three sequences are then selected and regions of interest are placed over liver parenchyma, excluding other elements to avoid the partial volume effect. The region of interest selection process for deMILI is illustrated in Figure 4. The NASHMRI and Fibro_MRI scores are then calculated by the software.

Resoundant

The vPDFF images are analysed using an automated post-processing tool (Hepatogram plus, Resoundant, Inc, Rochester, MN) to generate ROIs which are then reviewed and modified as needed by an expert reader. ROIs are drawn on 4 different slices, avoiding vessels, non-liver tissue, and susceptibility artifacts. Mean and range of liver fat fraction (%) and R2* (ms^{-1}), and ROI size are reported (Figure 5) (22).

Histology scoring

The quality assurance procedures relating to histology processing and scoring in LITMUS are described in detail in the protocol of the European NAFLD Registry(20) and some more details are provided in the supplementary methods.

Statistical analysis

The statistical analysis will be conducted using the complete data from the European NAFLD Registry as described above. The complete dataset will include details of the results from the analysis of the imaging biomarkers collected in the LITMUS Imaging study, and details of the reference standard and other biomarkers collected directly into the European NAFLD Registry.

The diagnostic accuracy of each imaging index test will be evaluated for each target condition and expressed as the area under the receiver operator characteristic curve (AUC). The reference standard will be based on liver histology, centrally read by consensus between two pathologists and scored according to the NASH CRN scoring system.

The following main target conditions will be defined using the centrally read histology scores:

1. NASH (NAS≥4, with at least 1 point in all the components) vs No-NASH
2. Significant fibrosis (F≥2
3. Advanced fibrosis (F≥3)
4. Cirrhosis (F4)
5. "NASH at risk of progression" (NASH + F≥2) vs. No-NASH or NASH + F<2
6. Cirrhosis with NASH (NASH +F4) vs. No-NASH or NASH + F<4

The steatosis grade (0-1 vs 2-3) and the grade of iron deposition (0 vs 1-4) will be secondary target conditions of interest.

The initial analyses will be performed using all available data. The AUC of each imaging index tests will be compared against the AUC of the FIB-4 index, a simple non-invasive test for fibrosis, and the AUC of liver stiffness measured by VCTE, each calculated in the same group of participants.

In sensitivity analyses, we will perform a direct comparison of the AUC of all imaging index tests, using standardization with weights for the availability of data, calculated with logistic regression based on age, sex, fibrosis stage and type II diabetes. To adjust for centre differences as potential confounders, we will also calculate country-adjusted ROC curves.

In addition, we will evaluate the performance of imaging biomarkers as screening tests, to be applied before biopsy for selecting those at high risk of the target condition. This context of use aims to reduce the screen failure rate in future drug trials in NAFLD, where the combination of significant fibrosis and active NASH is required for eligibility. This analysis will be based on the likelihood ratio of the imaging test result, derived from the kernel-smoothed cumulative distribution functions in those with and without the target condition.

Additional analyses for the diagnostic performance of combination biomarkers (e.g. FAST (23), MAST(24), cTAG (25)) and for the associations with other reference standards, like quantitative histology scores are planned using appropriate statistical methods.

Discussion

The LITMUS Imaging Study is the most ambitious study of its kind to date. The scope of the study is unique in terms of the high number of MR biomarkers included as well as the number of centres and participants contributing data. A unique strength of the LITMUS Imaging Study is its integration with the European NAFLD Registry which provides further opportunities for the exploration of biomarker combinations from the two studies. While data sets from the two studies are integrated, access to the entire dataset is limited only to the statistical analysis team. The imaging core labs quantifying the MR parameters are blinded from the clinical and histology data, and likewise the pathologists doing the central histology readings are blinded from the imaging biomarker results.

Beyond the cross-sectional evaluation of diagnostic accuracy of imaging biomarkers against liver histology, the LITMUS Imaging Study will investigate the natural history of NAFLD over a time horizon of 6 to 24 months. This could potentially identify biomarkers and their associations with disease progression or regression, something that will be useful in clinical care. Furthermore, the study will evaluate the confounding effect of a number of factors.

One potential limitation of the study is that given the slowly progressive nature of NAFLD, the maximum time interval of 24 months for the repeat assessment may still not long enough to establish the predictive potential of changes in imaging biomarkers. Furthermore, as there is no planned biopsy at the follow-up point, histological validation will not be possible at the time of the second scan. Despite these limitations, the follow-up data can still provide useful comparative data for how biomarkers change over time and whether they all move in the same direction. Furthermore, important insights can be gained by examining how biomarkers behave in relation to changes in other clinical parameters like weight.

Imaging tests have excellent reproducibility profiles and hence have great potential for implementation in clinical trials, as monitoring and response biomarkers and ultimately as surrogate endpoints. However, practical implementation has to overcome challenges with standardisation across sites, countries, scanner manufacturer and image analysis core labs. To address these challenges, the LITMUS Imaging Study has brought together key imaging experts from clinical, academic and industry stakeholders. This collaboration has led to the development of a liver imaging protocol that is being rigorously tested in this prospective study and that could produce data of sufficient quality for regulatory submissions, and methods that could be used in future clinical trials.

In summary, the LITMUS Imaging Study is a prospective cohort study evaluating imaging biomarkers of NAFLD using robust standardisation and quality control processes. The study will produce data that could ultimately be used to improve clinical outcomes, through biomarker qualifications or application of biomarkers in clinical practice.

Tables

Table 1: Overview of the MR acquisition protocol

MR Imaging Protocol Step	***Sequences***	***Target Endpoints***
Liver Multi Scan	LMS Base Slice LMS MOLLI LMS IDEAL LMS T2star Dixon	Iron-corrected T1 (ms) Perspectum PDFF (%) T2* (ms)
Vendor-specific T1 Mapping	MOLLI	Vendor-specific T1 (ms)
DeMILI	DEMILI TSE-T2-BH DEMILI STIR DEMILI 3D-FFE-T1 (DINAMYC)	NASH_MRI (0-1) Fibro_MRI (0-1)
Diffusion-Weighted MRI	Diffusion-weighted single-shot SE-EPI	ADC (mm^2/s)
Vendor-specific Liver PDFF	Multi-echo 3D GRE with vendor-specific PDFF reconstruction	Vendor-specific PDFF (%) and R2*(ms^{-1})
Magnetic Resonance Elastography	GRE MRE or SE-EPI MRE	Liver shear stiffness (kPa)

Figures

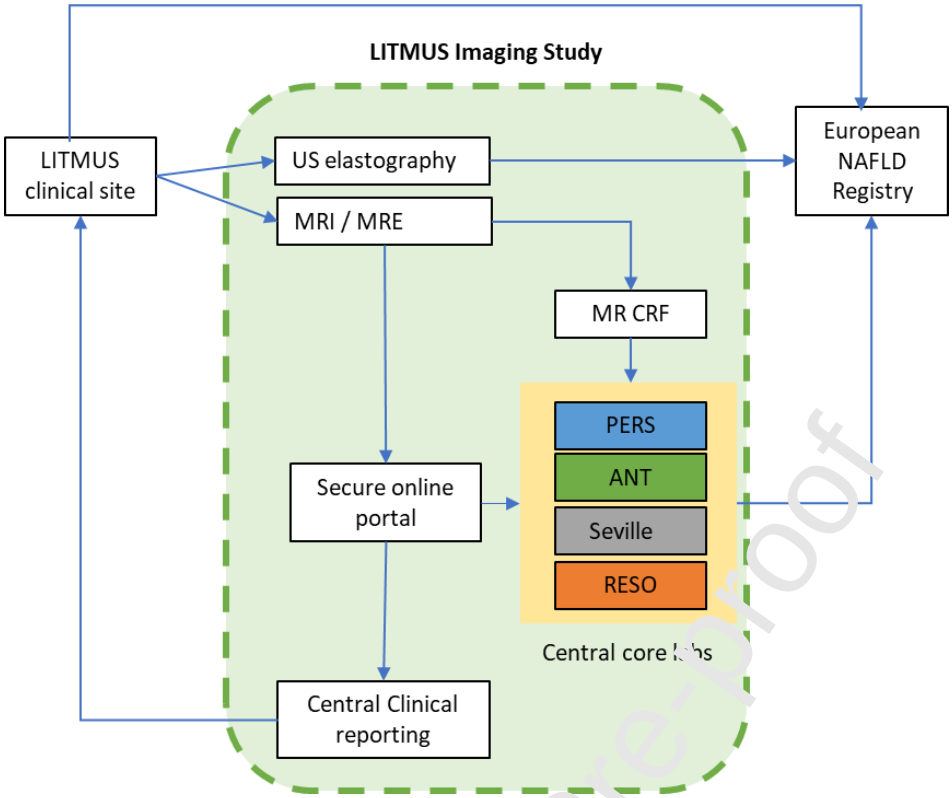

Figure 1. Data flow in the LITMUS Imaging Study.

Clinical sites in the LITMUS consortium recruit participants to the European NAFLD Registry study and to the LITMUS Imaging Study. Clinical data and biological samples are provided to the European NAFLD Registry. Participants in the LITMUS Imaging Study undergo ultrasound elastography and / or magnetic resonance scans. The results of ultrasound elastography are provided to the European NAFLD Registry directly from the recruiting site. Magnetic Resonance data are uploaded to a secure online portal and from there to four imaging core labs for central quantitative analysis. The results of this analysis are then provided to the European NAFLD Registry for integration with the rest of the data and downstream analysis.

Abbreviations: CRF: case record form; PERS: Perspectum core lab, ANT: Antaros Medical core lab, RESO: Resoundant core lab

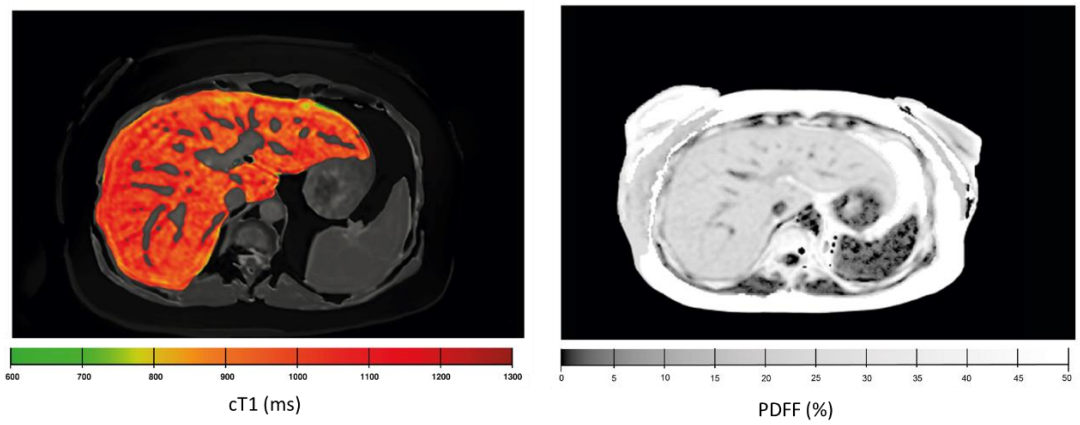

Figure 2. Sample data analysed by the Perspectum core lab

(a) Iron corrected T1 and (b) Perspectum PDFF analysis using Liver*MultiScan* (LMS). The illustrated patient has mean cT1 of 925ms and mean 22% PDFF. cT1 and PDFF metrics are obtained from whole liver segmentation maps of the MRI images. Non-parenchyma structures such as bile ducts and large blood vessels as well as image artifacts were excluded from image analysis.

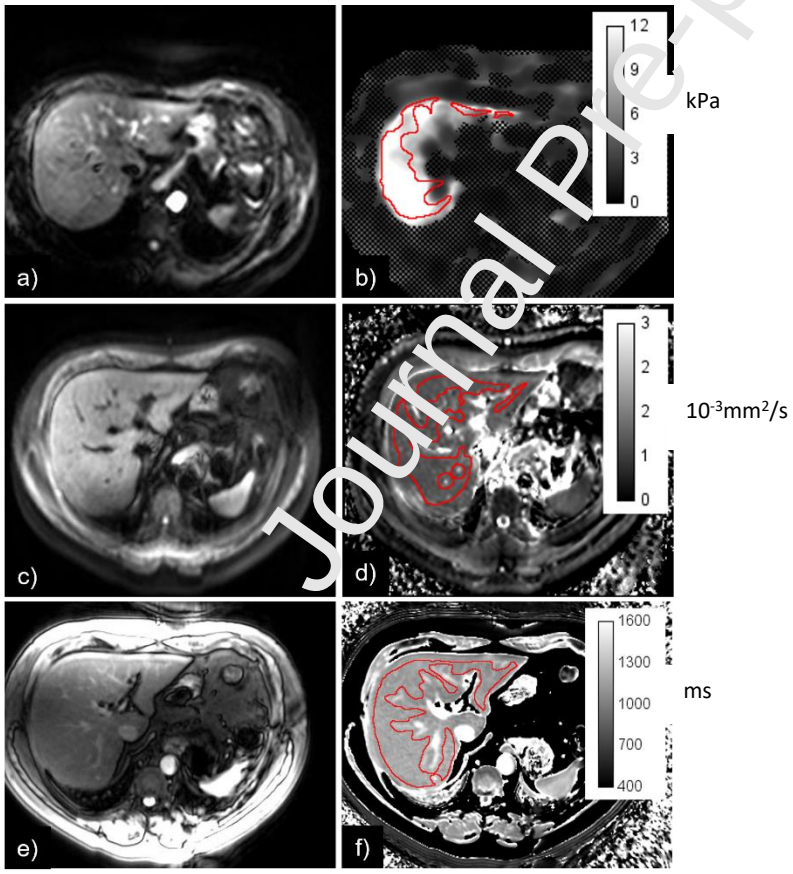

Figure 3. Sample data analysed by the Antaros core lab.

a) SE-EPI magnetic resonance elastography magnitude image. b) Shear stiffness map c) Diffusion-weighted image with b-value of 200 s/mm². d) Apparent diffusion coefficient map e) modified Look-Locker inversion recovery (MOLLI) image. f) Vendor T1 map.

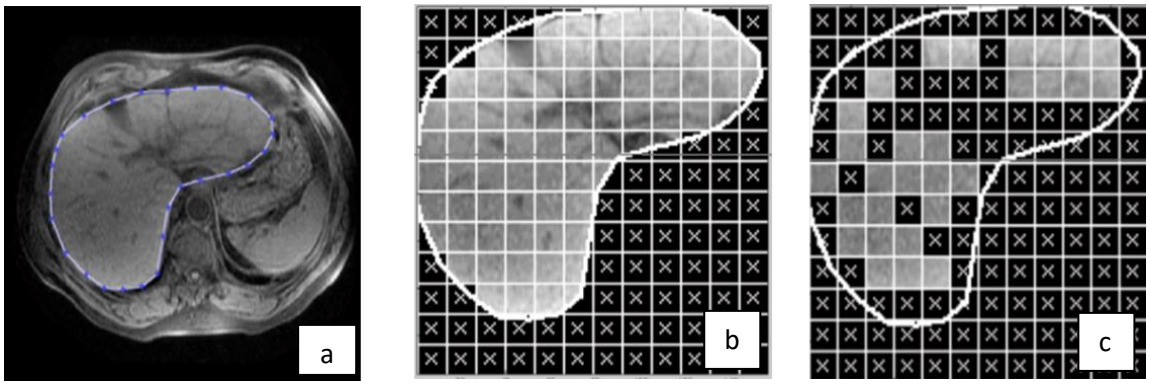

Figure 4. Sample data analysed by the Seville core lab.
The images illustrate the DeMILI region of interest selection process. (a) The liver is first segmented from axial slices and (b) overlaid to the regions of interest grid. (c) Regions of interest that include vessels or bile ducts or lie over the liver margin are exclude from analysis.

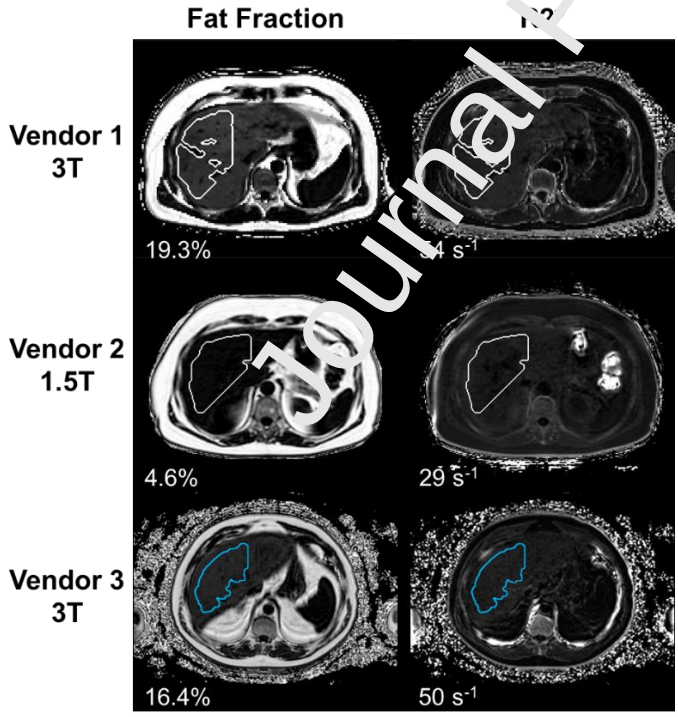

Figure 5: Sample data analysed by the Resoundant core lab.
Vendor-specific PDFF (vPDFF) sample images from 3 different subjects and MRI scanner manufacturers. Fat fraction images (left column) and R2* images (right column) with applied ROIs and corresponding mean values are shown.

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Conflict of Interest

FEM, SA, KW, JC, MA, EMG, SF, MRG, JC, IFL, RA, RSG, JMP, JB, VR, MW, SP, MA, RF, LM, ET, ME, PL, ATH, GP, HYJ, KP and PMB declare no conflicts of interest.

MP is a shareholder in Perspectum Ltd.

EMT is a shareholder in Perspectum Ltd.

SA is currently employed by Boehringer Ingelheim (but was not during his participation in the project)

GPA has served as a consultant and an advisory board member for Pfizer Inc, Inventiva Pharma, GlaxoSmithKline and KaNDy Therapeutics; he has been a consultant to BerGenBio ASA, Median Technologies, FRACTYL, Amryt Pharmaceuticals and AstraZeneca; and has given presentations on behalf of Roche Diagnostics and Medscape all through the University of Nottingham contract.

CA declares the following potential conflicts of interest: Hologic: Support of study and expert; Guerbet: Member of board, PI of study; Siemens: Expert.

EB has served as a consultant or advisory board member for Boehringer Ingelheim, Gilead Sciences, Intercept, Merck, Novo Nordisk, Pfizer, ProSciento; and a speaker for Gilead Sciences, Intercept, Merck, Novo Nordisk, Pfizer. She has also received a research grant from Gilead Sciences for fatty liver research.

AG served as a speaker and consultant for AbbVie, Alexion, AstraZeneca, Bayer, BMS, CSL Behring, Eisai, Falk, Gilead, Heel, Intercept, Ipsen, Merz, MSD, Novartis, Pfizer, Roche, Sanofi-Aventis, Sequana; received research funding from Intercept, Falk, Novartis.

JMS reports consultancy for BMS, Boehringer Ingelheim, Echosens, Genfit, Gilead Sciences, Intercept Pharmaceuticals, Madrigal, Novartis, Pfizer, Roche, Sanofi; received research funding from Gilead Sciences and was on the speaker's bureau for Falk Foundation MSD Sharp & Dohme GmbH.

MJS and **PH** are employed by Antaros Medical AB, Mölndal, Sweden.

ES is employed by Perspectum Ltd, Oxford, UK.

RB is a shareholder and employed (CEO) by Perspectum Ltd, Oxford, UK.

KP and **MK** are employed by Resoundant Inc, Rochester, MN, USA.

RLE and the Mayo Clinic have intellectual property rights and a financial interest in magnetic resonance elastography technology

AT is employed by ADVANZPHARMA, Capital House, 1st Floor, 85 King William Street, London, EC4N 7BL, United Kingdom

HC is employed by Boehringer Ingelheim Pharma GmbH & Co.

MM is employed by Novartis AG, Basel, Switzerland

CY is employed by Pfizer Inc., Lake Mary, FL, USA

TT was employed by Pfizer at the time of her involvement with the project.

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SN is a shareholder in Perspectum Ltd.

SAH has research grants from Akero, Altimmune, Axcella-Cirius, CiVi Biopharma, Cymabay, Galectin, Genfit, Gilead Sciences, Hepion Pharmaceuticals, Hightide Therapeutics, Intercept, Madrigal, Metacrine, NGM Bio, Northsea Therapeutics, Novartis, Novo Nordisk, Poxel, Sagimet, Viking. He has received consulting fees from Akero, Altimmune, Alentis, Arrowhead, Axcella, Echosens, Enyo,

Foresite Labs, Galectin, Genfit, Gilead Sciences, Hepion, HIghtide, HistoIndex, Intercept, Kowa, Madrigal, Metacrine, NeuroBo, NGM, Northsea, Novartis, Novo Nordisk, Poxel, Perspectum, Sagimet, Terns, and Viking.