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Global Research Initiative for Patient Screening on MASH (GRIPonMASH) protocol: rationale and design of a prospective multicenter study

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ABSTRACT

Introduction

The prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) may be as high as 38% in the adult population with potential serious complications, multiple co-morbidities and a high socioeconomic burden. However, there is a general lack of awareness and knowledge about MASLD and its progressive stages (MASH and fibrosis). Therefore, MASLD is still far underdiagnosed. The "Global Research Initiative for Patient Screening on MASH" (GRIPonMASH) consortium focuses on this unmet public health need. GRIPonMASH will help (primary) health care providers to implement a patient care pathway, as recommended by different scientific societies, to identify patients at risk of severe MASLD and to raise awareness. Furthermore, GRIPonMASH will contribute to a better understanding of the pathophysiology of MASLD and improved identification of diagnostic and prognostic markers to detect individuals at risk.

Methods

This is a prospective multicenter observational study in which 10.000 high risk patients (type 2 diabetes mellitus, obesity, metabolic syndrome or hypertension) will be screened in 10 European countries using at least two non-invasive tests (FIB-4 and FibroScan). Blood samples and liver biopsy material will be collected and biobanked, and multi-omics analyses will be conducted.

Ethics and dissemination

The study will be conducted in compliance with this protocol, ICH GCP, and applicable national and international regulatory requirements. The study initiation package is submitted at the local level. The study protocol has already been approved by local medical ethical committees in 5 of the 10 participating countries. Regulatory submission is ongoing in the remaining countries. Results will be made public and published in scientific, peer-reviewed, international journals and at international conferences.

Registration details

Clinicaltrials.gov ID: NCT05651724, registration date: 15 Dec 2022

Strenghts and limitations of this study

1. Multicenter European study embedded in clinical practice
2. Implementation of accepted European guidelines
3. Stimulate collaboration between primary and secondary care
4. Liver biopsies only in subset of patients

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) [1] is a disease with increasing prevalence, associated with metabolic and cardiovascular morbidity [2], [3], [4], [5]. The estimated global prevalence of MASLD is now over 38% [6]. Patients with comorbidities such as type 2 diabetes mellitus (T2DM), obesity, metabolic syndrome and hypertension are at an increased risk for MASLD. Prevalence estimates range up to i.e., 55% in people with diabetes and up to 75% for people with obesity [7], [8], [9]. Overall, the socioeconomic burden and costs of MASLD are high [10], [11]. Despite its prevalence and associated risks, MASLD is often underdiagnosed.

The progressive stages of the MASLD spectrum are metabolic dysfunction-associated steatohepatitis (MASH) and MASH with progressive liver fibrosis, which can eventually lead to liver cirrhosis, liver failure and hepatocellular carcinoma [12]. Approximately 20 to 25% of MASLD patients develop MASH. The estimated prevalence of MASH in the general European adult population is 6-7% [13]. Again, a much higher prevalence has been suggested in i.e., those with T2DM (up to 65%) and obesity (up to 30%) [14]. The severity of MASH is determined by the presence and stage of liver fibrosis, a key determinant of liver-related complications and both liver-related and all-cause mortality [4], [15]. The gold standard for a confirmatory MASH diagnosis and staging is histology by liver biopsy, although non-invasive tests (NITs) are increasingly being used as diagnostic and prognostic tools as well [16], [17].

MASLD is usually asymptomatic and is often not detected until patients enter the progressive stages and/or develop complications, whereas in the early stages the disease is reversible. In the USA, MASH is already the leading cause of liver transplants in women, and the second most common cause in men [18]. Therefore, timely diagnosis and staging of MASLD is important to identify the growing group of patients at increased risk of liver-related and cardiovascular complications. A multidisciplinary approach should be initiated in these patients, which may include intensive lifestyle intervention [19], participation in therapeutic trials and, in selected cases, bariatric surgery [20]. The first drug for this indication (Resmetirom) has been approved by the FDA in March 2024 [21].

Several scientific societies have proposed patient care pathways for implementation in clinical practice to identify patients with severe MASLD [22], [23], [24]. The European Association for the Study of Liver (EASL), the European Association for the Study of Diabetes (EASD) and the European Association for the Study of Obesity (EASO) jointly developed NAFLD clinical practice guidelines in 2016 [16]. In 2021 the first "*Clinical Practice Guidelines on Non-invasive tests for Evaluation of Liver Disease Severity and Prognosis*" were published [23], which proposed a diagnostic flow chart using concordant NITs to screen patients at risk. NITs are generally categorized as blood-based diagnostics (that can be either algorithms based on liver enzymes, or specific serum markers, usually combined) or imaging-based techniques (i.e., ultrasound, magnetic resonance imaging, transient elastography). In June 2024 an updated guideline was published: *"EASL-EASD-EASO Clinical Practice Guidelines on the*

management of metabolic dysfunction-associated steatotic liver disease (MASLD)" reflecting the new nomenclature and recent developments in the field [25]. While the aforementioned and multiple other international guidelines from scientific societies [16], [25], [26], [27], [28], urge to screen individuals at high risk of developing progressive stages of MASLD, these recommendations have not been broadly implemented [29], [30], [31]. There are several reasons for this: MASLD is a silent disease, there is lack of awareness among professionals for MASLD and its associated risks [32], and no pharmacological therapeutic intervention is available in Europe yet.

To assist (primary) health care providers to implement the patient care pathway as suggested by the EASL [23], [25], the "Global Research Initiative for Patient Screening on MASH" (GRIPonMASH) study has been designed. Within this study, high risk patients identified at primary care practices or attending hospital outpatient clinics will be screened for the presence of MASLD, liver fibrosis and (at-risk) MASH using at least 2 NITs in 10 European countries. Additional published and exploratory NITs will also be investigated [33]. Furthermore, a central biobank will be set up and multi-omics will be applied to gain a better understanding of the pathophysiology of MASLD-MASH, and to evaluate diagnostic and prognostic markers to identify patients at risk. Simultaneously, GRIPonMASH aims to promote awareness and implementation of screening among (primary) health care providers, thereby improving clinical care.

METHODS AND ANALYSIS

Study design

In this prospective multicenter, observational study the implementation of a transmural patient care pathway will be promoted. The study will initially employ 2 NITs: 1) the most commonly used blood-based diagnostic, the Fibrosis-4 index (FIB-4); and 2) liver stiffness measurement by vibration-controlled transient elastography (LSM by VCTE) using the FibroScan®. In total 10.000 adult patients at high risk to develop MASLD and MASH (i.e., having T2DM, metabolic syndrome, obesity or arterial hypertension) will be screened. See Fig. 1 for an overview of the study design.

Patient care pathway and return to standard care

Participants will be recruited at consultation hours at participating primary care centers and outpatient clinics. Prospective participants will be invited to the Center of Excellence (CoE) for the first visit. Informed consent will be obtained from all individual participants included in the study. Participants will be instructed to come to each visit after an overnight fast. The assessments of the first visit include baseline clinical characteristics, blood sampling, a FibroScan examination and a lifestyle assessment (by patient-reported outcome (PRO) surveys and an optional 24-hour dietary recall). The participants and referring (primary) health care providers will receive feedback on the FibroScan results and the next steps to take according to the patient care pathway. When the LSM by VCTE results are indeterminate (LSM 8 – 12 kPa) lifestyle recommendations will be provided to the participant.

The patient care pathway provides 3 management options based on the LSM by VCTE results. Option 1) if the FibroScan examination indicates low risk of advanced fibrosis (LSM < 8 kPa), the participant returns to standard care. Option 2) when the FibroScan examination is indeterminate (LSM 8 – 12 kPa), the FibroScan examination and blood sampling will be repeated after 12 weeks (visit 2). Depending on the re-test the participant either returns to standard care (if LSM < 8 kPa) or will be referred to a specialist at the MASLD clinic for detailed analysis (if LSM ≥ 8 kPa). Alternative diagnoses will be excluded as described in the EASL guideline [16], [25], blood will be sampled and a liver biopsy is offered to definitively diagnose presence of MASH and the fibrosis stage, following local clinical care protocols (visit 3). If MASH is not confirmed after detailed analysis, the participant will return to their referring care provider. If MASH is confirmed, the participant will stay at the MASLD clinic for follow-up and possible treatment according to the most recent local guidelines. Patients fulfilling the criteria to undergo bariatric surgery, should receive this recommendation if the procedure is available in the participating country/CoE. If during the study, pharmaceutical therapies become available for this indication, these should be prescribed following current local practice. Option 3) when the FibroScan examination indicates advanced fibrosis (LSM > 12 kPa), the participant will be referred to a specialist at the MASLD clinic for detailed analysis according to local clinical care protocols (visit 3). In these cases a liver biopsy is also strongly advised, unless there is already evidence of liver decompensation. The clinical follow-up of patients at the MASLD clinic is part of standard clinical care, including the local reading of liver biopsies.

Long-term follow-up

All participants will be included in a long-term follow-up program. The European guidelines recommend to retest patients at risk every 1-5 years [23], [25]. At 3 and 5 years after inclusion date, all participants are invited for a follow-up visit which includes a FibroScan examination, fasted blood sampling, PRO surveys and a follow-up questionnaire.

Study duration

The expected study duration is 8 years. After the pre-screening at the (P)HCP, a participant should attend the first visit at the CoE within 4 weeks. Within 2 weeks, the referring care provider should receive feedback from the CoE with the results of the first visit and recommendation for follow-up according to the patient care pathway. In the case of LSM > 12 kPa at visit 1, the participants should receive an appointment with the specialist at the MASLD clinic within 16 weeks for further detailed analysis and a possible liver biopsy. In the case of LSM ≥ 8 kPa at retest (visit 2), the participants should receive an appointment at with the specialist at the MASLD clinic within 30 weeks for further detailed analysis and a possible liver biopsy. If available, earlier consultation is allowed. Participants should be fully evaluated within a period of 8 months after referral and feedback needs to be sent to the referring care provider.

Study population

This study will be conducted in adult patients (over 18 years of age) with a current or prior diagnosis of at least one of the following four conditions: T2DM, metabolic syndrome, obesity or arterial hypertension. Participants must meet the following inclusion criteria: 1) being willing to provide written informed consent; 2) 18-75 years of age; 3) either diagnosed with or currently being treated for at least one of the earlier mentioned conditions according to the criteria provided in Table 1.

Table 1: Inclusion criteria, based on criteria for diagnosis of type 2 diabetes mellitus, obesity, arterial hypertension and metabolic syndrome. HCP = health care provider.

Condition	Criteria	
Type 2 diabetes mellitus	At least 2 times a fasting glucose > 7,0 mmol/L	
	Or elevated non-fasting glucose >11,1 mmol/L 2 hrs after OGT	
	Or HbA1c ≥48 mmol/mol (≥6.5%)	
	Or being actively treated for previously diagnosed type 2 diabetes by a HCP	
Obesity	Body mass index (BMI) ≥ 30 kg/m²	
	Or waist circumferences: *Caucasian: male ≥ 94 cm, female ≥ 80 cm* *South-Asian/Chinese: male ≥90 cm, female ≥80 cm* *Japanese: male ≥85 cm, female ≥90 cm*	
Arterial hypertension	Systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg	
	Or being actively treated for previously diagnosed arterial hypertension by a HCP	
Metabolic syndrome	Central obesity defined with waist circumference (see above) *if BMI is ≥30 kg/m², central obesity can be assumed and waist circumference does not need to be measured*	
	AND any two of the following:	
	Raised triglycerides	≥ 150 mg/dL (1.7 mmol/L) *or specific treatment for this lipid abnormality*
	Reduced HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in males < 50 mg/dL (1.29 mmol/L) in females *or specific treatment for this lipid abnormality*
	Raised blood pressure (BP)	Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg *or treatment of previously diagnosed hypertension*
	Raised fasting plasma glucose (FPG)	FGP ≥ 100 mg/dL (5.6 mmol/L) 2h OGTT ≥ 7.8 mmol/L or previously diagnosed type 2 diabetes *(if above >5.6 mmol/L or 100 mg/dL, an oral glucose tolerance test is strongly recommended, but is not necessary to define presence of the syndrome)*

A participant who meets any of the following criteria will be excluded from participation in this study: 1) patient with known hepatitis B, C or HIV or any other liver condition (like hemochromatosis, sarcoidosis, Wilson's disease, etc); 2) patient with any other condition that may lead to liver fibrosis or cirrhosis; 3) patient engages in (excessive) alcohol use (defined as: > 3 units/day [30 grams/day] in males and > 2 units/day [20 grams/day] in females; 4) patient with history or evidence of any other clinically significant condition or planned or expected procedure that in the opinion of the investigator, may compromise the patient's safety or ability to be included in this study; 5) the patient is an

employee or contractor of the facility that is conducting the study or is a family member of the investigator, sub-Investigator, or any sponsor personnel; 6) the patient is not able to understand the details of the protocol and/or is not able to provide written informed consent; 7) the patient is pregnant or breastfeeding; 8) the patient underwent bariatric surgery in the last 12 months.

Study sites

The 10 selected countries are: Belgium, Czech Republic, France, Germany, Greece, Italy, Portugal, Romania, Spain and The Netherlands. In each country clinics defined as CoE and several satellite primary care centers will be recruited. The general aim is to recruit 1-2 CoE per country depending on the local size and distribution of primary care centers and CoEs. CoEs should fulfil the following criteria: 1) CoE should be able to perform liver biopsies; 2) CoE should have personnel trained to carry out FibroScan examinations. XL probe needs to be available to be used when indicated by FibroScan; 3) CoE should be able to store blood and liver samples for a limited time; 4) CoE should be able to arrange shipment of samples by courier services to the central biobank; 5) CoE should have sufficient access to (at least 2) primary care centers willing to participate in GRIPonMASH including at least 100 patients per practice; 6) the team of CoE should at least comprise: 1 internist/diabetologist or endocrinologist; 1 gastroenterologist/hepatologist.

Biobank

The collected liver and blood samples will be shipped to a central biobank at UMC Utrecht, the Netherlands. Participants will be asked to approve of biobanking of their blood, DNA and liver samples for a total of 15 years, since we foresee rapid developments in available diagnostic tools and multi-omics methods. Some samples will be used for pre-specified central assessments. We aim to analyse the remaining samples over the next decade using any novel scientific advances.

Sample size estimation

The primary objectives of this study are to establish the prevalence of MASLD, at-risk MASH and liver fibrosis based on FibroScan examinations in high-risk patients, to compare the prevalence between countries and assess the added value of a 2-step pathway (see Table 4). As such, this study should be considered a pilot study helping to implement clinical guidelines and consolidate patient care pathways. We estimate that including 1000 patients per country will give a realistic reflection of the situation per country and that it will provide enough power to determine differences between included countries and diagnostics (see Figure 2).

Study procedures

Randomisation, blinding and treatment allocation will not occur in this study, as participants will follow the flow of the patient care pathway. Treatment of T2DM, metabolic syndrome, obesity and/or arterial hypertension will continue according to routine care.

Baseline characteristics

At the first visit the following data will be recorded: age, gender, ethnicity, primary diagnosis for inclusion and date of diagnosis, comorbidities, short medical history, short family history and current medication use. A short physical exam will be performed including: height, weight, waist circumference and blood pressure. If the participant does not recall medication use, the investigator will request this information from the care provider. Results of recent (not older than 6 months) laboratory measurements of urine albumin/creatinine ratio and presence of microalbuminuria will be requested from the care provider, if available.

FibroScan examination

All patients will undergo a FibroScan (Echosens, France) examination to assess simultaneously LSM by VCTE and controlled attenuation parameter (CAP™). The measurements will be carried out in participants fasting for at least three hours and after a 5-minute resting time in the supine position on the examination bed by a trained and certified staff member of the CoE. Tables 2 and 3 provide the cut-off values that will be used to interpret LSM and CAP respectively. CAP can be used to estimate steatosis and LSM to estimate fibrosis. Type of probe used and validity of measurements will also be recorded.

Table 2: cut-off values for controlled attenuation parameter (CAP) [25], [34], [35].

CAP (dB/m)	Indication of steatosis stage
248 – 267	S1
268 – 279	S2
≥ 280	S3

Table 3: cut-off values for liver stiffness measurement (LSM) by vibration-controlled transient elastography [25], [35], [36].

LSM by VCTE (kPa)	Indication fibrosis stage
< 8	Low risk of advanced fibrosis
8 – 12	Indeterminate risk
> 12	Advanced fibrosis

The recommendations for the next steps in the patient care pathways are based on the LSM by VCTE results, which provides an indication of the fibrosis stage of the participant. The referring care provider will be informed about the results and recommendations following a predefined format.

Blood sampling and processing

All blood samples will be collected after an overnight fast. At visit 1, 47 mL of blood will be drawn an processed to allow biobanking, direct central analysis and local measurements. 6 mL blood, 4 plasma aliquots and 8 serum aliquots will be biobanked. 1 serum aliquot and 2 mL blood in a PAX tube will be shipped directly to central analysis. The CoE will perform local measurements of aspartate aminotransferase (AST) level, alanine transaminase (ALT) level, platelet count and Hba1c. At visit 2, 8 mL blood will be drawn an processed into 1 plasma aliquot and 2 serum aliquots for biobanking and 1 PAX tube for central analysis. At visit 3 and the follow-up visits, 14 mL blood will be drawn and processed into 2 plasma aliquots and 3 serum aliquots for biobanking and 1 PAX tube for central

analysis. Each CoE will receive 'biobank kits' including tubes and pre-printed labels to ensure high-quality sample collection that is comparable across sites. The samples processed for biobanking will be stored locally at -80 degrees Celsius and then be shipped from the CoE to the central biobank in batches.

Fibrosis-4 index (FIB-4)

The FIB-4 is a widely used blood-based diagnostic [23], [37], [38]. The FIB-4 index is an algorithm based on age, AST, ALT and platelet count and is calculated at baseline using the following formula: FIB-4 = (AGE (years) * AST (U/L)) / (platelet count (10^9/L) * square root(ALT (U/L))).

FibroScan-AST (FAST) score

The FibroScan-AST (FAST) score is an algorithm combining the FibroScan examination results (LSM by VCTE and CAP) with a blood marker (AST) to identify patients with active fibrotic-MASH, also referred to as at-risk MASH [39].

Liver biopsy

Liver biopsies will be carried out according to local procedures at the participating CoE. The CoE will prepare: 1) slides for local reading according to local standard operating procedures; 2) three unstained slides to allow central reading and digital pathology assessment; and 3) snap freeze liver tissue with liquid nitrogen to allow metabolomic analysis. These tissue samples will be temporarily stored at the CoE before shipment to the central biobank. A panel of 2 independent and well-trained pathologists will execute the central reading. The histopathological features of MASH (steatosis, hepatocyte ballooning, lobular inflammation) will be assessed using the NAS criteria [40] and Steatosis, Activity, and Fibrosis (SAF) score [41], and the fibrosis stage will be determined.

Baseline laboratory measurements (central)

The following variables will be measured centrally for each participant. Metabolic panel: total cholesterol, triglycerides, HDL-C, LDL-C, ApoB, glucose, creatinine. Liver enzymes: ALT, AST, bilirubin (direct and total), gamma-glutamyltransferase, alkaline phosphatase. Other: Lp(a), insulin, SHBG, FT4, T3, TSH and albumin.

Genetic and proteomic analysis (central)

DNA will be extracted from the biobanked full blood samples. Genetic analyses of patients will be accomplished by genome-wide association studies (GWAS). Targeted and untargeted proteomics analysis will be used to identify serological protein biomarkers.

Metabolomic, lipidomic and fluxomic analysis (central)

Metabolomics, lipidomics and fluxomics studies will be performed on the liver biopsy and fasted blood samples. Metabolites including lipids will be extracted from EDTA plasma samples and analysed using mass-spectrometry (MS) based metabolomics and lipidomics methods. A global metabolomics high-

resolution platform will be used allowing the targeted and untargeted/global analyses of a wide range of hundreds of small molecules including amino acids, central energy and carbon metabolism, but also exogenous molecules dietary chemicals, microbiome-derived metabolites, environmental chemicals, commercial products, and drugs. A lipidomics platform allows the analysis of more than 20 lipid classes including diglycerides, triglycerides, phospholipids, ceramides, sphingolipids and many more. A targeted MS/MS platform will be used covering more than 300 modified free fatty acids acting as bioactive lipid mediators both locally and systemically which are involved in immunology (innate immunity), inflammation, chemotaxis, cell survival, cell proliferation and differentiation. Fluxomic measurement based on metabolites labelled e.g., with 13C-glucose or 2H2O will be performed in vitro and in vivo to quantify metabolic fluxes and elucidate pathophysiological mechanisms.

Biomarker analysis (central)

For the identification of novel circulating biomarkers, a panel of (potential) biomarkers will be identified in vitro by using liver-on-a-chip tools. Additionally, potential novel biomarkers (i.e., PLIN-2 [HeparDx], PRO-C3, PRO-C6, oxidized LDL, glucagon, GIP, GLP-1, FICE34, TLM3) will be measured.

Lifestyle phenotyping

Lifestyle will be phenotyped in 7 different areas: diet, water consumption, alcohol consumption, physical activity, smoking, sleep and endocrine environmental disruptors. The following self-reported questionnaires are used: diet will be assessed using the Mediterranean Diet Score (PREDIMED) [42]; water consumption will be obtained with one question; alcohol consumption, apart from wine in the PREDIMED, will be obtained with a maximum of 3 semi-closed questions; physical activity will be recorded using the International Physical Activity Questionnaire – Short Form (IPAQ-SF) [43]; smoking habits will be obtained with a maximum of 8 multiple and semi-closed question; sleep quality will be obtained with Brief version Pittsburgh Sleep Quality Index (B-PSQI) [44]; endocrine disrupting chemical exposures will be evaluated using 6 questions. Additionally, in CoEs with a dietician and resources available, a 24-hour dietary recall will be annotated by an interviewer on 2 days (most recent regular weekday and one weekend day).

Lifestyle recommendations

Lifestyle recommendations will be provided based on current clinical guidelines for MASLD/MASH patients [25], [45]. A member of the study team will recommend participants adhere to a healthy dietary pattern and level of physical activity. The aim of the lifestyle recommendations is to lose 5% weight (ideally 10%); however, losing weight should not be the participant's ultimate goal but rather their adherence to a healthy lifestyle.

Long-term follow-up (at 3 and 5 years)

A follow-up questionnaire has been designed to inquire about the disease status and comorbidities, possible liver-related and non-liver related complications and changes in medication of the participant. The first follow-up visit will be at 3 years, where the FibroScan examination will be repeated. At the

same visit, the follow-up questionnaire should be completed by the research team who will request the necessary information from the participant's care provider. The second follow-up visit will be at 5 years, where the care provider will be asked to complete the follow-up questionnaire again. At both timepoints the subjects will be asked to complete the PRO surveys again.

Table 4: primary and secondary objectives, subobjectives and associated outcome measures.

PRIMARY OBJECTIVE		
Objective	**Sub-objectives**	**Outcome measures**
To implement a transmural patient care pathway, to identify patients with MASLD and its progressive form of MASH in primary care centers and clinics in 10 European countries	Prevalence of liver steatosis and MASLD estimated by FibroScan CAP in patients at risk	- Steatosis stage deduced from CAP measurement with FibroScan - MASLD definition (CAP > 280 and 1 out of 5 cardiometabolic risk factors)
	Prevalence of liver fibrosis estimated by FibroScan LSM in patients at risk	Fibrosis stage deduced from LSM by VCTE measurement with FibroScan
	Prevalence of at-risk MASH estimated by FAST score in patients at risk	At-risk MASH deduced from FAST score
	Prevalence of MASH in patients at risk (in subset only)	MASH diagnosis confirmed by histology (NAS/SAF criteria) upon liver biopsy
	Comparison of the prevalence of liver steatosis, MASLD, liver fibrosis and (at-risk) MASH between the participating countries	Prevalence (see above) stratified per country
	Added value of a 2-step pathway (FIB-4 + FibroScan) as compared to FibroScan only for detection of high-risk patients	Number of patients at risk identified by FIB-4 in comparison to numbers found using LSM by VCTE with FibroScan, and numbers found in combination
SECONDARY OBJECTIVES		
Objective	**Sub-objectives (if applicable)**	**Outcome measures**
To gain a better understanding of the pathophysiology of MASLD and to identify markers that will help to detect patients at risk (by applying a multi-omics approach)	Build/validate a diagnostic model to identify MASH patients in a high-risk population	Possible model parameters are all baseline clinical characteristics reported in the eCRF
	Explore genotypes related to MASH in different European countries	Genomic (GWAS) and proteomics analysis on collected blood samples
	Explore (non-invasive) metabolite biomarkers identifying MASH in patients at risk	Mass-spectrometry (MS) based metabolomic and lipidomic analyses and fluxomics analysis on collected blood samples, both targeted and untargeted approaches
	Prevalence of co-morbidities and associated therapies (especially for cardiovascular disease) in patients with MASH compared to those without, in high risk patient populations.	Prevalence of comorbidities in patients at- risk, patients diagnosed with MASLD/MASH
	Identify prognostic factors/biomarkers for complications in patients with MASLD and MASH at 5 years	Disease progression and liver-related and non-liver related complications
Evaluate Patient Reported Outcomes (PRO) from baseline throughout follow-up		PRO surveys at baseline and follow-up
Confirm high risk of liver fibrosis based on second FibroScan examination after lifestyle recommendations (in subset only)		Revisited FibroScan examination (Cap and LSM by VCTE) 12 weeks after first FibroScan and after lifestyle recommendations
To evaluate changes in CAP, LSM by VCTE and FAST scores over time		Repeated CAP, LSM by VCTE and FAST measurement over time

Data management

Clinical data will be captured using an electronic case report form (eCRF), only authorised staff at the CoE will be allowed to enter data into the eCRF. All eCRF data should be verifiable to a source at the CoE or accessible by the site staff, except if direct entry was allowed. Participants are identified by a code, only the local investigators will have the key. The investigator(s) will be responsible for ensuring eCRF data completeness and accuracy. Source data verification and data quality will be assessed by remote eCRF reviews, statistical checks and in-person monitor visits. Data from the eCRF and other sources (i.e., central analyses, Liver Health Management platform) will be encoded and stored in a central study database (Data Science Platform).

Statistical analysis

R will be used for statistical analysis, p-values < 0.05 (2-tailed) are considered statistically significant. By default, parametric testing will be employed. In case of not normally distributed variables, non-parametric tests will be employed. Before analysis, we will assess the degree and possible reasons for missingness and apply appropriate methods to handle missing data i.e., multiple imputation.

As the primary objective is the implementation of a patient care pathway (see table 4), primarily descriptive statistics will be used instead of hypothesis testing. Prevalence of MASLD, liver steatosis, fibrosis and (at-risk) MASH will be reported for each country. The prevalence will be compared between countries using one-way ANOVA (or Kruskal Wallis test). The overlap in numbers of patients at risk identified by the 2 NITs will be reported as well.

For the secondary objectives the following statistical methods will be used: diagnostic modelling using logistic regression and machine learning (ML)/artificial intelligence (AI) approaches; associations in GWAS and proteomics data will be analysed using logistic regression; associations between MASH diagnosis by histology and (non-invasive) metabolite (bio)markers will be analysed using logistic regression; prevalence of co-morbidities will be estimated in total group of patients at risk with/without MASLD, the difference between the groups will be analysed using one-way ANOVA (or Kruskal Wallis test); prognostic modelling using logistic regression and ML/AI approaches; mixed linear modelling will be implemented to assess changes in PRO over time; difference between 1st and 2nd LSM by VCTE measurements will be assessed using a paired t-test (or Wilcoxon signed rank test); mixed linear modelling will be implemented to assess the repeated measurements of CAP, LSM by VCTE and FAST over time.

Ethics and dissemination

The study will be conducted in compliance with this protocol, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), and applicable national and international regulatory requirements (including Declaration of Helsinki). The study initiation package is submitted at the local level. The study protocol has already been approved by local medical ethical committees in the following countries: Netherlands (MEC-U, A23.273/R22.057), Germany (Etik-Kommission

Landesartzekammer Rheinland-Pfalz, 2022-16602), Spain (CEI de los Hospitales Universitarios Virgen Macarena y Virgen del Rocío, 1631-N-22), Belgium (Ethische Commissie Universitair Ziekenhuis Antwerpen, EDGE 002626) and Portugal (Comissao de Etica do CAML, 203/23). Regulatory submission is being initiated in the remaining countries. Results will be made public and published in scientific, peer-reviewed, international journals and at international conferences.

Patient and Public Involvement

The draft protocol was reviewed and endorsed by Liver Patients International (LPI). The European Liver Patients Association (ELPA), the European Atherosclerosis Society (EAS) and the European Association for the Study of the Liver (EASL) have also endorsed the final project.

DISCUSSION

Although several factors are involved in the progression from MASLD to MASH (environmental, dietary, genetic), the exact mechanisms behind MASLD development and progression remain unknown [46]. The widely used estimate of 38% worldwide prevalence of MASLD is based on a meta-analysis of studies from 2016 to 2019 [6]. However, exact data about the prevalence in different European countries using accepted diagnostic tools like LSM by VCTE or the golden standard, liver biopsy, are still lacking. This study will provide prevalence estimates based on a large number of participants.

The patient care pathway used in this study is based on the 2021 and 2024 updates of the EASL clinical practice guidelines [23], [25]. In contrast to these guidelines, in the current protocol the two NITs (FIB-4 and FibroScan) will be performed in the same visit. Recently there has been debate about the accuracy of FIB-4 to (pre-)screen in the general population at primary care level [47], as the index was validated and developed in secondary and tertiary care. This issue was acknowledged by the EASL in 2021 and they already advised not to base diagnoses on one single NIT, but to use concordant NITs instead [23]. Therefore, in the current protocol it was decided to perform the FibroScan and blood sampling for FIB-4 at the same day for each patient. We can then compare the added value of FIB-4 versus FibroScan and their prognostic capacity at follow up within a population at risk identified at the primary care level and outpatient clinics, and thereby either validate the guidelines or propose adjustments.

In line with the EASL Clinical Practice Guidelines, structured programmes aimed at lifestyle changes towards healthy diet and habitual physical activity are advisable [16]. The current study only includes lifestyle recommendations to a subset of participants as this would be feasible within current clinical practice. The level of adherence to the Mediterranean diet will be evaluated throughout the study. The Mediterranean diet is thought to be beneficial [48], although evidence is still limited [49]. We also aim to build towards personalized lifestyle advice.

European experts have demonstrated that most countries in Europe are not yet ready to face the growing MASLD/MASH problem, because they lack guidelines, registries and multidisciplinary programs [30]. Therefore, initiatives such as this study aimed at increasing knowledge and awareness of MASLD and MASH among professionals are essential. In addition, this study aims to integrate all levels of clinical care, which is much needed for multidisciplinary management of MASLD.

Author contributions

DEG and MCC conceptualized the study. VDJ and MCC were the main authors of the manuscript and protocol. DEG, OHF and the members of the initial Julius Clinical-initiated Scientific Steering Committee (HCP, SF, CM, MRG, MET, AGH, LS, JMS, JWMM, AG, VR) and Independent Advisory Board (JW, MA) critically reviewed the initial protocol. TH contributed to the lipidomics/metabolomics section, JV/MD to the liver biopsy section. CFP, RB, GVD, LM, VR, DCS critically review the revised protocol.

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Competing interests statement

JMS: Consultant: Akero, Alentis Therapeutics, Astra Zeneca, 89Bio, Boehringer Ingelheim, GSK, Ipsen, Inventiva Pharma, Madrigal, MSD, Northsea Therapeutics, Novartis, Novo Nordisk, Pfizer, Roche, Sanofi, Siemens Healthineers. Research Funding: Boehringer Ingelheim, Siemens Healthcare GmbH. Stock Options: AGED diagnostics, Hepta Bio. Speaker Honorarium: Gilead Sciences, Advanz, Echosens, MedPublico GmbH.

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AG: consultancy to Boehringer Ingelheim, Eli Lilly and Company, Metadeq Diagnostics; has participated in advisory boards for: Boehringer Ingelheim, Merck Sharp & Dohme, Novo Nordisk, Metadeq Diagnostics and Pfizer; speaker honorarium or other fees from: Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk and Pfizer.

The other authors have no relevant financial or non-financial interests to disclose.

Provenance and peer review

The protocol was peer reviewed by the members of the initial Julius Clinical-initiated GRIPonMASH Scientific Steering Committee and Principal Investigators of the CoEs. The authors are thankful to Prof. J. Ordovas for providing critical feedback on the final version of this manuscript.

Data availability statement

Collected data will be available upon reasonable request. Only unidentified data are available to researchers who submit a methodologically sound proposal. Proposals may be submitted to the GRIPonMASH consortium for review. The consortium reserves the right to analyse data for development and intellectual property purposes prior to public disclosure.

Figure legends

Fig 1: Overview of study design and patient flow. Abbreviations used: (P)HCP = (primary) health care provider, PIF = patient information folder, ICF = informed consent form, FIB-4 = Fibrosis-4 index, LSM = liver stiffness measurement by VCTE.

Fig 2: Expected patient flow through patient care pathway. Based on estimates available in literature, in the total at risk population of 10.000 participants, we expect 80% to score LSM < 8 kPa (low risk), 17% is expected to score LSM 8 – 12 kPa (indeterminate) and 3% LSM > 12 kPa (advanced fibrosis) upon screening with FibroScan. We assume at least 80% will have LSM ≥ 8 kPa at the re-test. Of those who will be offered liver biopsy, we expect that for 70% MASH diagnosis will be confirmed upon biopsy. In theory this leads to approximately 950 biopsy confirmed diagnoses of MASH. However, considering reluctance for invasive testing and loss to follow-up, we expect to identify approximately 500 confirmed MASH patients in this study, and thus on average 50 per country.

REFERENCES

[1] M. E. Rinella et al., 'A multi-society Delphi consensus statement on new fatty liver disease nomenclature.', Hepatology, Jun. 2023, doi: 10.1097/HEP.0000000000000520.

[2] C. D. Byrne and G. Targher, 'NAFLD: a multisystem disease', J Hepatol, vol. 62, no. 1 Suppl, pp. S47–S64, 2015, doi: 10.1016/J.JHEP.2014.12.012.

[3] G. Targher, C. D. Byrne, A. Lonardo, et al., 'Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis', J Hepatol, vol. 65, no. 3, pp. 589–600, Sep. 2016, doi: 10.1016/J.JHEP.2016.05.013.

[4] M. Ekstedt et al., 'Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up', Hepatology, vol. 61, no. 5, pp. 1547–1554, May 2015, doi: 10.1002/HEP.27368.

[5] S. Driessen et al., 'Metabolic dysfunction associated steatotic liver disease and the heart', Hepatology, Dec. 2023, doi: 10.1097/HEP.0000000000000735.

[6] Z. M. Younossi, P. Golabi, J. M. Paik, et al., 'The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review.', Hepatology, Jan. 2023, doi: 10.1097/HEP.0000000000000004.

[7] J. Quek et al., 'Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis', Lancet Gastroenterol Hepatol, vol. 8, no. 1, pp. 20–30, Jan. 2023, doi: 10.1016/S2468-1253(22)00317-X.

[8] L. Henry, J. Paik, and Z. M. Younossi, 'Review article: the epidemiologic burden of non-alcoholic fatty liver disease across the world', Aliment Pharmacol Ther, vol. 56, no. 6, pp. 942–956, Sep. 2022, doi: 10.1111/APT.17158.

[9] Z. M. Younossi et al., 'The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis', J Hepatol, vol. 71, no. 4, pp. 793–801, Oct. 2019, doi: 10.1016/J.JHEP.2019.06.021.

[10] J. M. Schattenberg et al., 'Disease burden and economic impact of diagnosed non-alcoholic steatohepatitis in five European countries in 2018: A cost-of-illness analysis', Liver Int, vol. 41, no. 6, pp. 1227–1242, Jun. 2021, doi: 10.1111/LIV.14825.

[11] T. H. Karlsen et al., 'The EASL–Lancet Liver Commission: protecting the next generation of Europeans against liver disease complications and premature mortality', The Lancet, vol. 399, no. 10319, pp. 61–116, Jan. 2022, doi: 10.1016/S0140-6736(21)01701-3/ATTACHMENT/82513520-DAB3-430E-A196-83F57E6FC6C3/MMC1.PDF.

[12] E. E. Powell, V. W. S. Wong, and M. Rinella, 'Non-alcoholic fatty liver disease', Jun. 05, 2021, Elsevier B.V. doi: 10.1016/S0140-6736(20)32511-3.

[13] Z. M. Younossi, A. B. Koenig, D. Abdelatif et al., 'Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes', Hepatology, vol. 64, no. 1, pp. 73–84, Jul. 2016, doi: 10.1002/HEP.28431/SUPPINFO.

[14] J. F. Dufour et al., 'The global epidemiology of nonalcoholic steatohepatitis (NASH) and associated risk factors–A targeted literature review', Endocrine and Metabolic Science, vol. 3, p. 100089, Jun. 2021, doi: 10.1016/J.ENDMTS.2021.100089.

[15] A. J. Sanyal et al., 'Prospective Study of Outcomes in Adults with Nonalcoholic Fatty Liver Disease', N Engl J Med, vol. 385, no. 17, pp. 1559–1569, Oct. 2021, doi: 10.1056/NEJMOA2029349.

[16] G. Marchesini et al., 'EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease', J Hepatol, vol. 64, no. 6, pp. 1388–1402, Jun. 2016, doi: 10.1016/J.JHEP.2015.11.004.

[17] F. E. Mózes et al., 'Performance of non-invasive tests and histology for the prediction of clinical outcomes in patients with non-alcoholic fatty liver disease: an individual participant data meta-analysis', Lancet Gastroenterol Hepatol, vol. 8, no. 8, pp. 704–713, Aug. 2023, doi: 10.1016/S2468-1253(23)00141-3.

[18] M. Noureddin et al., 'NASH Leading Cause of Liver Transplant in Women: Updated Analysis of Indications For Liver Transplant and Ethnic and Gender Variances', Am J Gastroenterol, vol. 113, no. 11, pp. 1649–1659, Nov. 2018, doi: 10.1038/S41395-018-0088-6.

[19] V. Ratziu, 'Non-pharmacological interventions in non-alcoholic fatty liver disease patients', Liver International, vol. 37, pp. 90–96, Jan. 2017, doi: 10.1111/LIV.13311.

[20] M. Ren, X. Zhou, L. Lv, et al., 'Endoscopic Bariatric and Metabolic Therapies for Liver Disease: Mechanisms, Benefits, and Associated Risks', J Clin Transl Hepatol, vol. 10, no. 5, p. 986, Oct. 2022, doi: 10.14218/JCTH.2021.00448.

[21] S. A. Harrison et al., 'A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis', N Engl J Med, vol. 390, no. 6, pp. 497–509, Feb. 2024, doi: 10.1056/NEJMOA2309000.

[22] F. Kanwal et al., 'Clinical Care Pathway for the Risk Stratification and Management of Patients With Nonalcoholic Fatty Liver Disease', Gastroenterology, vol. 161, no. 5, pp. 1657–1669, Nov. 2021, doi: 10.1053/J.GASTRO.2021.07.049.

[23] A. Berzigotti et al., 'EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update', J Hepatol, vol. 75, no. 3, pp. 659–689, Sep. 2021, doi: 10.1016/J.JHEP.2021.05.025.

[24] A. Srivastava et al., 'Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease', J Hepatol, vol. 71, no. 2, pp. 371–378, Aug. 2019, doi: 10.1016/J.JHEP.2019.03.033.

[25] Association for the Study of the Liver et al., 'EASL–EASD–EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD)', J Hepatol, vol. 0, no. 0, Jun. 2024, doi: 10.1016/J.JHEP.2024.04.031.

[26] M. E. Rinella et al., 'AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease.', Hepatology, Feb. 2023, doi: 10.1097/HEP.0000000000000323.

[27] K. Cusi et al., 'American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD)', Endocrine Practice, vol. 28, no. 5, pp. 528–562, May 2022, doi: 10.1016/J.EPRAC.2022.03.010.

[28] P. Ferenci et al., 'World Gastroenterology Organisation Global Guidelines Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis', 2014. [Online]. Available: www.jcge.com

[29] Q. M. Anstee et al., 'Real-world management of non-alcoholic steatohepatitis differs from clinical practice guideline recommendations and across regions', JHEP Reports, vol. 4, no. 1, Jan. 2022, doi: 10.1016/j.jhepr.2021.100411.

[30] J. v. Lazarus et al., 'European "NAFLD Preparedness Index" — Is Europe ready to meet the challenge of fatty liver disease?', JHEP Reports, vol. 3, no. 2, p. 100234, Apr. 2021, doi: 10.1016/J.JHEPR.2021.100234.

[31] J. M. Schattenberg, Q. M. Anstee, C. Caussy, et al., 'Differences between current clinical guidelines for screening, diagnosis and management of nonalcoholic fatty liver disease and real-world practice: a targeted literature review', 2021, Taylor and Francis Ltd. doi: 10.1080/17474124.2021.1974295.

[32] S. Driessen et al., 'A global survey of health care workers' awareness of non-alcoholic fatty liver disease: The AwareNASH survey', United European Gastroenterol J, 2023, doi: 10.1002/UEG2.12445.

[33] Y. Vali et al., 'Biomarkers for staging fibrosis and non-alcoholic steatohepatitis in non-alcoholic fatty liver disease (the LITMUS project): a comparative diagnostic accuracy study', Lancet Gastroenterol Hepatol, vol. 8, no. 8, pp. 714–725, Aug. 2023, doi: 10.1016/S2468-1253(23)00017-1.

[34] P. J. Eddowes et al., 'Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease', Gastroenterology, vol. 156, no. 6, pp. 1717–1730, May 2019, doi: 10.1053/J.GASTRO.2019.01.042.

[35] V. W. S. Wong et al., 'Unified interpretation of liver stiffness measurement by M and XL probes in non-alcoholic fatty liver disease', Gut, vol. 68, no. 11, pp. 2057–2064, 2019, doi: 10.1136/GUTJNL-2018-317334.

[36] W. Theel et al., 'Evaluation of nonalcoholic fatty liver disease (NAFLD) in severe obesity using noninvasive tests and imaging techniques', Obesity Reviews, vol. 23, no. 8, p. e13481, Aug. 2022, doi: 10.1111/OBR.13481.

[37] J. v Lazarus et al., 'Real-world evidence on non-invasive tests and associated cut-offs used to assess fibrosis in routine clinical practice', JHEP Reports, vol. 0, no. 0, p. 100596, Sep. 2022, doi: 10.1016/J.JHEPR.2022.100596.

[38] E. J. Grobbee et al., 'Improvement of non-invasive tests of liver steatosis and fibrosis as indicators for non-alcoholic fatty liver disease in type 2 diabetes mellitus patients with elevated cardiovascular risk profile using the PPAR-α/γ agonist aleglitazar', PLoS One, vol. 17, no. 11, p. e0277706, Nov. 2022, doi: 10.1371/JOURNAL.PONE.0277706.

[39] P. N. Newsome et al., 'FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study', Lancet Gastroenterol Hepatol, vol. 5, no. 4, pp. 362–373, Apr. 2020, doi: 10.1016/S2468-1253(19)30383-8.

[40] A. C. Sheka, O. Adeyi, J. Thompson, et al., 'Nonalcoholic Steatohepatitis: A Review', JAMA, vol. 323, no. 12, pp. 1175–1183, Mar. 2020, doi: 10.1001/JAMA.2020.2298.

[41] F. Nascimbeni et al., 'Clinical validation of the FLIP algorithm and the SAF score in patients with non-alcoholic fatty liver disease', J Hepatol, vol. 72, no. 5, pp. 828–838, May 2020, doi: 10.1016/J.JHEP.2019.12.008.

[42] M. A. Martínez-González et al., 'A 14-Item Mediterranean Diet Assessment Tool and Obesity Indexes among High-Risk Subjects: The PREDIMED Trial', PLoS One, vol. 7, no. 8, Aug. 2012, doi: 10.1371/JOURNAL.PONE.0043134.

[43] C. L. Craig et al., 'International physical activity questionnaire: 12-country reliability and validity', Med Sci Sports Exerc, vol. 35, no. 8, pp. 1381–1395, Aug. 2003, doi: 10.1249/01.MSS.0000078924.61453.FB.

[44] C. Sancho-Domingo, J. L. Carballo, A. Coloma-Carmona et al., 'Brief version of the Pittsburgh Sleep Quality Index (B-PSQI) and measurement invariance across gender and age in a population-based sample', Psychol Assess, vol. 33, no. 2, pp. 111–121, Feb. 2021, doi: 10.1037/PAS0000959.

[45] S. M. Francque et al., 'Non-alcoholic fatty liver disease: A patient guideline'.

[46] Q. M. Anstee et al., 'Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort☆', J Hepatol, vol. 73, no. 3, pp. 505–515, Sep. 2020, doi: 10.1016/J.JHEP.2020.04.003.

[47] I. Graupera et al., 'Low Accuracy of FIB-4 and NAFLD Fibrosis Scores for Screening for Liver Fibrosis in the Population', Clin Gastroenterol Hepatol, vol. 20, no. 11, pp. 2567-2576.e6, Nov. 2022, doi: 10.1016/J.CGH.2021.12.034.

[48] Y. Gepner et al., 'The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content', J Hepatol, vol. 71, no. 2, pp. 379–388, Aug. 2019, doi: 10.1016/J.JHEP.2019.04.013.

[49] M. C. P. Torres et al., 'Mediterranean Diet and NAFLD: What We Know and Questions That Still Need to Be Answered.', Nutrients, vol. 11, no. 12, pp. E2971–E2971, Dec. 2019, doi: 10.3390/NU11122971.

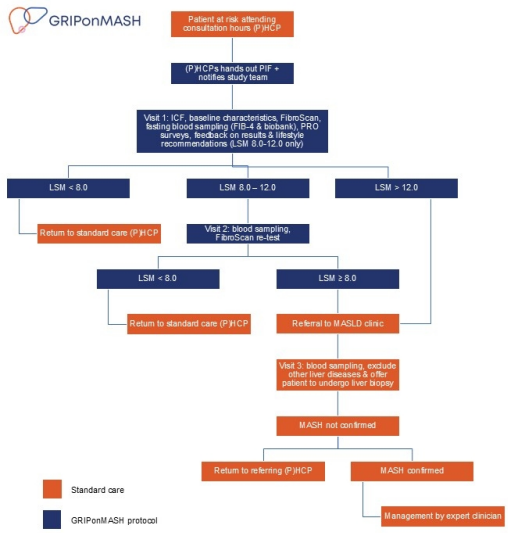

Fig 1: Overview of study design and patient flow. Abbreviations used: (P)HCP = (primary) health care provider, PIF = patient information folder, ICF = informed consent form, FIB-4 = Fibrosis-4 index, LSM = liver stiffness measurement by VCTE.

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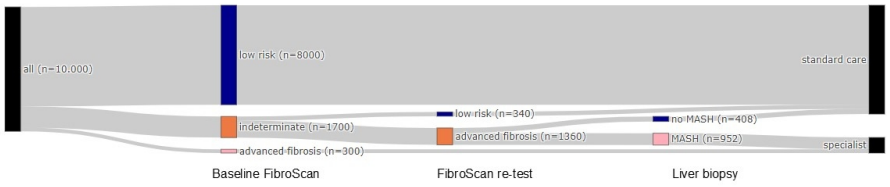

Fig 2: Expected patient flow through patient care pathway. Based on estimates available in literature, in the total at risk population of 10.000 participants, we expect 80% to score LSM < 8 kPa (low risk), 17% is expected to score LSM 8 – 12 kPa (indeterminate) and 3% LSM > 12 kPa (advanced fibrosis) upon screening with FibroScan. We assume at least 80% will have LSM ≥ 8 kPa at the re-test. Of those who will be offered liver biopsy, we expect that for 70% MASH diagnosis will be confirmed upon biopsy. In theory this leads to approximately 950 biopsy confirmed diagnoses of MASH. However, considering reluctance for invasive testing and loss to follow-up, we expect to identify approximately 500 confirmed MASH patients in this study, and thus on average 50 per country.

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Global Research Initiative for Patient Screening on MASH protocol (GRIPonMASH): rationale and design of a prospective multicenter study

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Global Research Initiative for Patient Screening on MASH protocol (GRIPonMASH): rationale and design of a prospective multicenter study

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ABSTRACT

Introduction

The prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) may be as high as 38% in the adult population with potential serious complications, multiple co-morbidities and a high socioeconomic burden. However, there is a general lack of awareness and knowledge about MASLD and its progressive stages (MASH and fibrosis). Therefore, MASLD is still far underdiagnosed. The "Global Research Initiative for Patient Screening on MASH" (GRIPonMASH) consortium focuses on this unmet public health need. GRIPonMASH will help (primary) health care providers to implement a patient care pathway, as recommended by multiple scientific societies, to identify patients at risk of severe MASLD and to raise awareness. Furthermore, GRIPonMASH will contribute to a better understanding of the pathophysiology of MASLD and improved identification of diagnostic and prognostic markers to detect individuals at risk.

Methods

This is a prospective multicenter observational study in which 10.000 high risk patients (type 2 diabetes mellitus, obesity, metabolic syndrome or hypertension) will be screened in 10 European countries using at least two non-invasive tests (FIB-4 and FibroScan). Blood samples and liver biopsy material will be collected and biobanked, and multi-omics analyses will be conducted.

Ethics and dissemination

The study will be conducted in compliance with this protocol, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), and applicable national and international regulatory requirements. The study initiation package is submitted at the local level. The study protocol has been approved by local medical ethical committees in all 10 participating countries. Results will be made public and published in scientific, peer-reviewed, international journals and at international conferences.

Registration details

Clinicaltrials.gov ID: NCT05651724, registration date: 15 Dec 2022

Strenghts and limitations of this study

1. Multicenter European study embedded in clinical practice
2. Implementation of accepted European guidelines
3. Stimulate collaboration between primary and secondary care
4. Liver biopsies only in subset of patients

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) [1] is a disease with increasing prevalence, associated with metabolic and cardiovascular morbidity [2], [3], [4], [5]. The estimated global prevalence of MASLD is now over 38% [6]. Patients with comorbidities such as type 2 diabetes mellitus (T2DM), obesity, metabolic syndrome and hypertension are at an increased risk for MASLD. Prevalence estimates range up to i.e., 55% in people with T2DM and up to 75% for people with obesity [7], [8], [9]. Overall, the socioeconomic burden and costs of MASLD are high [10], [11]. Despite its prevalence and associated risks, MASLD is often underdiagnosed.

The progressive stages of the MASLD spectrum are metabolic dysfunction-associated steatohepatitis (MASH) and MASH with progressive liver fibrosis, which can eventually lead to liver cirrhosis, liver failure and hepatocellular carcinoma [12]. Approximately 20 to 25% of MASLD patients develop MASH. The estimated prevalence of MASH in the general European adult population is 6-7% [13]. Again, a much higher prevalence has been suggested in i.e., those with T2DM (up to 65%) and obesity (up to 30%) [14]. The severity of MASH is determined by the presence and stage of liver fibrosis, a key determinant of liver-related complications and both liver-related and all-cause mortality [4], [15]. The gold standard for a confirmatory MASH diagnosis and staging is histology by liver biopsy, although non-invasive tests (NITs) are increasingly being used as diagnostic and prognostic tools as well [16], [17].

MASLD is usually asymptomatic and is often not detected until patients enter the progressive stages and/or develop complications, whereas in the early stages the disease is reversible. In the USA, MASH is already the leading cause of liver transplants in women, and the second most common cause in men [18]. Therefore, timely diagnosis and staging of MASLD is important to identify the growing group of patients at increased risk of liver-related and cardiovascular complications. A multidisciplinary approach should be initiated in these patients, which may include intensive lifestyle intervention [19], participation in therapeutic trials and, in selected cases, bariatric surgery [20]. The first drug for this indication (Resmetirom) has been approved by the FDA in March 2024 [21].

Several scientific societies have proposed patient care pathways for implementation in clinical practice to identify patients with severe MASLD [22], [23], [24]. The European Association for the Study of Liver (EASL), the European Association for the Study of Diabetes (EASD) and the European Association for the Study of Obesity (EASO) jointly developed NAFLD clinical practice guidelines in 2016 [16]. In 2021 the first "*Clinical Practice Guidelines on Non-invasive tests for Evaluation of Liver Disease Severity and Prognosis*" were published [23], which proposed a diagnostic flow chart using concordant NITs to screen patients at risk. NITs are generally categorized as blood-based diagnostics (that can be either algorithms based on liver enzymes, or specific serum markers, usually combined) or imaging-based techniques (i.e., ultrasound, magnetic resonance imaging, transient elastography). In June 2024 an updated guideline was published: *"EASL-EASD-EASO Clinical Practice Guidelines on the*

management of metabolic dysfunction-associated steatotic liver disease (MASLD)" reflecting the new nomenclature and recent developments in the field [25]. While the aforementioned and multiple other international guidelines from scientific societies [16], [25], [26], [27], [28], urge to screen individuals at high risk of developing progressive stages of MASLD, these recommendations have not been broadly implemented [29], [30], [31]. There are several reasons for this: MASLD is a silent disease, there is lack of awareness among professionals for MASLD and its associated risks [32], and no pharmacological therapeutic intervention is available in Europe yet.

To assist (primary) health care providers to implement the patient care pathway as suggested by the EASL [23], [25], the "Global Research Initiative for Patient Screening on MASH" (GRIPonMASH) study has been designed. Within this study, high risk patients identified at primary care practices or attending hospital outpatient clinics will be screened for the presence of MASLD, liver fibrosis and (at-risk) MASH using at least 2 NITs in 10 European countries. Additional published and exploratory NITs will also be investigated [33]. Furthermore, a central biobank will be set up and multi-omics will be applied to gain a better understanding of the pathophysiology of MASLD-MASH, and to evaluate diagnostic and prognostic markers to identify patients at risk. Simultaneously, GRIPonMASH aims to promote awareness and implementation of screening among (primary) health care providers, thereby improving clinical care.

METHODS AND ANALYSIS

Study design

In this prospective multicenter, observational study the implementation of a transmural patient care pathway will be promoted. The study will initially employ 2 NITs: 1) the most commonly used blood-based diagnostic, the Fibrosis-4 index (FIB-4); and 2) liver stiffness measurement by vibration-controlled transient elastography (LSM by VCTE) using the FibroScan®. In total 10.000 adult patients at high risk to develop MASLD and MASH (i.e., having T2DM, metabolic syndrome, obesity or arterial hypertension) will be screened. See Figure 1 for an overview of the study design.

Patient care pathway and return to standard care

Participants will be recruited at consultation hours at participating primary care centers and outpatient clinics. Prospective participants will be invited to the Center of Excellence (CoE) for the first visit. Informed consent will be obtained from all individual participants included in the study (Master Patient Information File included as Supplemental Material II). Participants will be instructed to come to each visit after an overnight fast. The assessments of the first visit include baseline clinical characteristics, blood sampling, a FibroScan examination and a lifestyle assessment (by patient-reported outcome (PRO) surveys and an optional 24-hour dietary recall). The participants and referring (primary) health care providers will receive feedback on the FibroScan results and the next steps to take according to the patient care pathway. When the LSM by VCTE results are indeterminate (LSM 8 – 12 kPa) lifestyle recommendations will be provided to the participant.

The patient care pathway provides 3 management options based on the LSM by VCTE results. Option 1) if the FibroScan examination indicates low risk of advanced fibrosis (LSM < 8 kPa), the participant returns to standard care. Option 2) when the FibroScan examination is indeterminate (LSM 8 – 12 kPa), the FibroScan examination and blood sampling will be repeated after 12 weeks (visit 2). Depending on the re-test the participant either returns to standard care (if LSM < 8 kPa) or will be referred to a specialist at the MASLD clinic for detailed analysis (if LSM ≥ 8 kPa). Alternative diagnoses will be excluded as described in the EASL guideline [16], [25], blood will be sampled and a liver biopsy is offered to definitively diagnose presence of MASH and the fibrosis stage, following local clinical care protocols (visit 3). If MASH is not confirmed after detailed analysis, the participant will return to their referring care provider. If MASH is confirmed, the participant will stay at the MASLD clinic for follow-up and possible treatment according to the most recent local guidelines. Patients fulfilling the criteria to undergo bariatric surgery, should receive this recommendation if the procedure is available in the participating country/CoE. If during the study, pharmaceutical therapies become available for this indication, these should be prescribed following current local practice. Option 3) when the FibroScan examination indicates advanced fibrosis (LSM > 12 kPa), the participant will be referred to a specialist at the MASLD clinic for detailed analysis according to local clinical care protocols (visit 3). In these cases a liver biopsy is also strongly advised, unless there is already evidence of liver decompensation. The clinical follow-up of patients at the MASLD clinic is part of standard clinical care, including the local reading of liver biopsies.

Long-term follow-up

All participants will be included in a long-term follow-up program. The European guidelines recommend to retest patients at risk every 1-5 years [23], [25]. At 3 and 5 years after inclusion date, all participants are invited for a follow-up visit which includes a FibroScan examination, fasted blood sampling, lifestyle assessment and a follow-up questionnaire.

Study duration

The expected study duration is 8 years, from June 2023 to March 2031. After the pre-screening at the (primary) health care provider, a participant should attend the first visit at the CoE within 4 weeks. Within 2 weeks, the referring care provider should receive feedback from the CoE with the results of the first visit and recommendation for follow-up according to the patient care pathway. In the case of LSM > 12 kPa at visit 1, the participants should receive an appointment with the specialist at the MASLD clinic within 16 weeks for further detailed analysis and a possible liver biopsy. In the case of LSM ≥ 8 kPa at retest (visit 2), the participants should receive an appointment at with the specialist at the MASLD clinic within 30 weeks for further detailed analysis and a possible liver biopsy. If available, earlier consultation is allowed. Participants should be fully evaluated within a period of 8 months after referral and feedback needs to be sent to the referring care provider.

Study population

This study will be conducted in adult patients (over 18 years of age) with a current or prior diagnosis of at least one of the following four conditions: T2DM, metabolic syndrome, obesity or arterial hypertension. Participants must meet the following inclusion criteria: 1) being willing to provide written informed consent; 2) 18-75 years of age; 3) either diagnosed with or currently being treated for at least one of the earlier mentioned conditions according to the criteria provided in Table 1.

Table 1: Inclusion criteria, based on criteria for diagnosis of type 2 diabetes mellitus, obesity, arterial hypertension and metabolic syndrome. HCP = health care provider.

Condition	Criteria	
Type 2 diabetes mellitus	At least 2 times a fasting glucose > 7,0 mmol/L	
	Or elevated non-fasting glucose >11,1 mmol/L 2 hrs after OGT	
	Or HbA1c ≥48 mmol/mol (≥6.5%)	
	Or being actively treated for previously diagnosed type 2 diabetes by a HCP	
Obesity	Body mass index (BMI) ≥ 30 kg/m²	
	Or waist circumferences: *Caucasian: male ≥ 94 cm, female ≥ 80 cm* *South-Asian/Chinese: male ≥90 cm, female ≥80 cm* *Japanese: male ≥85 cm, female ≥90 cm*	
Arterial hypertension	Systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg	
	Or being actively treated for previously diagnosed arterial hypertension by a HCP	
Metabolic syndrome	Central obesity defined with waist circumference (see above) *if BMI is ≥30 kg/m², central obesity can be assumed and waist circumference does not need to be measured*	
	AND any two of the following:	
	Raised triglycerides	≥ 150 mg/dL (1.7 mmol/L) *or specific treatment for this lipid abnormality*
	Reduced HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in males < 50 mg/dL (1.29 mmol/L) in females *or specific treatment for this lipid abnormality*
	Raised blood pressure (BP)	Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg *or treatment of previously diagnosed hypertension*
	Raised fasting plasma glucose (FPG)	FGP ≥ 100 mg/dL (5.6 mmol/L) 2h OGTT ≥ 7.8 mmol/L or previously diagnosed type 2 diabetes *(if above >5.6 mmol/L or 100 mg/dL, an oral glucose tolerance test is strongly recommended, but is not necessary to define presence of the syndrome)*

A participant who meets any of the following criteria will be excluded from participation in this study: 1) patient with known hepatitis B, C or HIV or any other liver condition (like hemochromatosis, sarcoidosis, Wilson's disease, etc); 2) patient with any other condition that may lead to liver fibrosis or cirrhosis; 3) patient engages in (excessive) alcohol use (defined as: > 3 units/day [30 grams/day] in males and > 2 units/day [20 grams/day] in females; 4) patient with history or evidence of any other clinically significant condition or planned or expected procedure that in the opinion of the investigator, may compromise the patient's safety or ability to be included in this study; 5) the patient is an

employee or contractor of the facility that is conducting the study or is a family member of the investigator, sub-Investigator, or any sponsor personnel; 6) the patient is not able to understand the details of the protocol and/or is not able to provide written informed consent; 7) the patient is pregnant or breastfeeding; 8) the patient underwent bariatric surgery in the last 12 months.

Study sites

The 10 selected countries are: Belgium, Czech Republic, France, Germany, Greece, Italy, Portugal, Romania, Spain and The Netherlands. In each country clinics defined as CoE and several satellite primary care centers will be recruited. The general aim is to recruit 1-2 CoE per country depending on the local size and distribution of primary care centers and CoEs. CoEs should fulfil the following criteria: 1) CoE should be able to perform liver biopsies; 2) CoE should have personnel trained to carry out FibroScan examinations. XL probe needs to be available to be used when indicated by FibroScan; 3) CoE should be able to store blood and liver samples for a limited time; 4) CoE should be able to arrange shipment of samples by courier services to the central biobank; 5) CoE should have sufficient access to (at least 2) primary care centers willing to participate in GRIPonMASH including at least 100 patients per practice; 6) the team of CoE should at least comprise: 1 internist/diabetologist or endocrinologist; 1 gastroenterologist/hepatologist.

Biobank

The collected liver and blood samples will be shipped to a central biobank at UMC Utrecht, the Netherlands. Participants will be asked to approve of biobanking of their blood, DNA and liver samples for a total of 15 years, since we foresee rapid developments in available diagnostic tools and multi-omics methods. Some samples will be used for pre-specified central assessments. We aim to analyse the remaining samples over the next decade using any novel scientific advances.

Sample size estimation

The primary objectives of this study are to establish the prevalence of MASLD, at-risk MASH and liver fibrosis based on FibroScan examinations in high-risk patients, to compare the prevalence between countries and assess the added value of a 2-step pathway (see Table 4). As such, this study should be considered a pilot study helping to implement clinical guidelines and consolidate patient care pathways. We estimate that including 1000 patients per country will give a realistic reflection of the situation per country and that it will provide enough power to determine differences between included countries and diagnostics (see Figure 2).

Study procedures

Randomisation, blinding and treatment allocation will not occur in this study, as participants will follow the flow of the patient care pathway. Treatment of T2DM, metabolic syndrome, obesity and/or arterial hypertension will continue according to routine care.

Baseline characteristics

At the first visit the following data will be recorded: age, gender, ethnicity, primary diagnosis for inclusion and date of diagnosis, comorbidities, short medical history, short family history and current medication use. A short physical exam will be performed including: height, weight, waist circumference and blood pressure. If the participant does not recall medication use, the investigator will request this information from the care provider. Results of recent (not older than 6 months) laboratory measurements of urine albumin/creatinine ratio and presence of microalbuminuria will be requested from the care provider, if available.

FibroScan examination

All patients will undergo a FibroScan (Echosens, France) examination to assess simultaneously LSM by VCTE and controlled attenuation parameter (CAP™). The measurements will be carried out in participants fasting for at least three hours and after a 5-minute resting time in the supine position on the examination bed by a trained and certified staff member of the CoE. Tables 2 and 3 provide the cut-off values that will be used to interpret LSM and CAP respectively. CAP can be used to estimate steatosis and LSM to estimate fibrosis. Type of probe used and validity of measurements will also be recorded.

Table 2: cut-off values for controlled attenuation parameter (CAP) [25], [34], [35].

CAP (dB/m)	Indication of steatosis stage
248 – 267	S1
268 – 279	S2
≥ 280	S3

Table 3: cut-off values for liver stiffness measurement (LSM) by vibration-controlled transient elastography [25], [35], [36].

LSM by VCTE (kPa)	Indication fibrosis stage
< 8	Low risk of advanced fibrosis
8 – 12	Indeterminate risk
> 12	Advanced fibrosis

The recommendations for the next steps in the patient care pathways are based on the LSM by VCTE results, which provides an indication of the fibrosis stage of the participant. The referring care provider will be informed about the results and recommendations following a predefined format.

Blood sampling and processing

All blood samples will be collected after an overnight fast. At visit 1, 47,5 mL of blood will be drawn an processed to allow biobanking, direct central analysis and local measurements. 6 mL blood, 4 plasma aliquots and 7 serum aliquots will be biobanked. 2 serum aliquots and 2 mL blood in a PAXgene tube will be shipped directly to central analysis. The CoE will perform local measurements of aspartate aminotransferase (AST) level, alanine transaminase (ALT) level, platelet count and Hba1c. At visit 2, 8,5 mL blood will be drawn an processed into 1 plasma aliquot and 2 serum aliquots for biobanking and 1 PAXgene tube for central analysis. At visit 3 and the follow-up visits, 16,5 mL blood will be drawn and processed into 2 plasma aliquots and 3 serum aliquots for biobanking and 1 PAXgene tube

for central analysis. Each CoE will receive 'biobank kits' including tubes and pre-printed labels to ensure high-quality sample collection that is comparable across sites. The samples processed for biobanking will be stored locally at -80 degrees Celsius and then be shipped from the CoE to the central biobank in batches.

Fibrosis-4 index (FIB-4)

The FIB-4 is a widely used blood-based diagnostic [23], [37], [38]. The FIB-4 index is an algorithm based on age, AST, ALT and platelet count and is calculated at baseline using the following formula: FIB-4 = (AGE (years) * AST (U/L)) / (platelet count (10^9/L) * square root(ALT (U/L))).

FibroScan-AST (FAST) score

The FibroScan-AST (FAST) score is an algorithm combining the FibroScan examination results (LSM by VCTE and CAP) with a blood marker (AST) to identify patients with active fibrotic-MASH, also referred to as at-risk MASH [39].

Liver biopsy

Liver biopsies will be carried out according to local procedures at the participating CoE. The CoE will prepare: 1) slides for local reading according to local standard operating procedures; 2) three unstained slides to allow central reading and digital pathology assessment; and 3) snap freeze liver tissue with liquid nitrogen to allow metabolomic analysis. These tissue samples will be temporarily stored at the CoE before shipment to the central biobank. A panel of 2 independent and well-trained pathologists will execute the central reading. The histopathological features of MASH (steatosis, hepatocyte ballooning, lobular inflammation) will be assessed using the NAS criteria [40] and Steatosis, Activity, and Fibrosis (SAF) score [41], and the fibrosis stage will be determined.

Baseline laboratory measurements (central)

The following variables will be measured centrally for each participant. Metabolic panel: total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), glucose, creatinine. Liver enzymes: alanine transaminase (ALT), aspartate aminotransferase (AST), bilirubin (direct and total), gamma-glutamyltransferase, alkaline phosphatase. Other: lipoprotein (a) (Lp(a)), insulin, sex hormone-binding globulin (SHBG), free thyroxine (FT4), tri-iodothyronine (T3), thyroid stimulating hormone (TSH), albumin and globulins.

Genetic and proteomic analysis (central)

DNA will be extracted from the biobanked full blood samples. Genetic analyses of patients will be accomplished by genome-wide association studies (GWAS). Targeted and untargeted proteomics analysis will be used to identify serological protein biomarkers.

Metabolomic, lipidomic and fluxomic analysis (central)

Metabolomics, lipidomics and fluxomics studies will be performed on the liver biopsy and fasted blood samples. Metabolites including lipids will be extracted from EDTA plasma samples and analysed using mass-spectrometry (MS) based metabolomics and lipidomics methods. A global metabolomics high-resolution platform will be used allowing the targeted and untargeted/global analyses of a wide range of hundreds of small molecules including amino acids, central energy and carbon metabolism, but also exogenous molecules dietary chemicals, microbiome-derived metabolites, environmental chemicals, commercial products, and drugs. A lipidomics platform allows the analysis of more than 20 lipid classes including diglycerides, triglycerides, phospholipids, ceramides, sphingolipids and many more. A targeted MS/MS platform will be used covering more than 300 modified free fatty acids acting as bioactive lipid mediators both locally and systemically which are involved in immunology (innate immunity), inflammation, chemotaxis, cell survival, cell proliferation and differentiation. Fluxomic measurement based on metabolites labelled e.g., with 13C-glucose or 2H2O will be performed in vitro and in vivo to quantify metabolic fluxes and elucidate pathophysiological mechanisms.

Biomarker analysis (central)

For the identification of novel circulating biomarkers, a panel of (potential) biomarkers will be identified in vitro by using liver-on-a-chip tools. Additionally, potential novel biomarkers (i.e., PLIN-2 [HeparDx], PRO-C3, PRO-C6, oxidized low-density lipoprotein [LDL], glucagon, gastric inhibitory polypeptide [GIP], glucagon-like peptide-1 [GLP-1], FICE34, TLM3) will be measured.

Lifestyle phenotyping

Lifestyle will be phenotyped in 7 different areas: diet, water consumption, alcohol consumption, physical activity, smoking, sleep and endocrine environmental disruptors. The following self-reported questionnaires are used: diet will be assessed using the Mediterranean Diet Score (PREDIMED) [42]; water consumption will be obtained with one question; alcohol consumption, apart from wine in the PREDIMED, will be obtained with a maximum of 3 semi-closed questions; physical activity will be recorded using the International Physical Activity Questionnaire – Short Form (IPAQ-SF) [43]; smoking habits will be obtained with a maximum of 8 multiple and semi-closed question; sleep quality will be obtained with Brief version Pittsburgh Sleep Quality Index (B-PSQI) [44]; endocrine disrupting chemical exposures will be evaluated using 6 questions. Additionally, in CoEs with a dietician and resources available, a 24-hour dietary recall will be annotated by an interviewer on 2 days (most recent regular weekday and one weekend day).

Lifestyle recommendations

Lifestyle recommendations will be provided based on current clinical guidelines for MASLD/MASH patients [25], [45]. A member of the study team will recommend participants adhere to a healthy dietary pattern and level of physical activity. The aim of the lifestyle recommendations is to lose 5% weight (ideally 10%); however, losing weight should not be the participant's ultimate goal but rather their adherence to a healthy lifestyle.

Long-term follow-up (at 3 and 5 years)

A follow-up questionnaire has been designed to inquire about the disease status and comorbidities, possible liver-related and non-liver related complications and changes in medication of the participant. At the follow-up visits at 3 and 5 years the FibroScan examination will be repeated. At the same visit, the follow-up questionnaire should be completed by the research team who will request the necessary information from the participant's care provider. At both timepoints the participants will be asked to complete the lifestyle assessment again.

Table 4: primary and secondary objectives, subobjectives and associated outcome measures.

PRIMARY OBJECTIVE		
Objective	**Sub-objectives**	**Outcome measures**
To implement a transmural patient care pathway, to identify patients with MASLD and its progressive form of MASH in primary care centers and clinics in 10 European countries	Prevalence of liver steatosis and MASLD estimated by FibroScan CAP in patients at risk	- Steatosis stage deduced from CAP measurement with FibroScan - MASLD definition (CAP > 280 and 1 out of 5 cardiometabolic risk factors)
	Prevalence of liver fibrosis estimated by FibroScan LSM in patients at risk	Fibrosis stage deduced from LSM by VCTE measurement with FibroScan
	Prevalence of at-risk MASH estimated by FAST score in patients at risk	At-risk MASH deduced from FAST score
	Prevalence of MASH in patients at risk (in subset only)	MASH diagnosis confirmed by histology (NAS/SAF criteria) upon liver biopsy
	Comparison of the prevalence of liver steatosis, MASLD, liver fibrosis and (at-risk) MASH between the participating countries	Prevalence (see above) stratified per country
	Added value of a 2-step pathway (FIB-4 + FibroScan) as compared to FibroScan only for detection of high-risk patients	Number of patients at risk identified by FIB-4 in comparison to numbers found using LSM by VCTE with FibroScan, and numbers found in combination
SECONDARY OBJECTIVES		
Objective	**Sub-objectives (if applicable)**	**Outcome measures**
To gain a better understanding of the pathophysiology of MASLD and to identify markers that will help to detect patients at risk (by applying a multi-omics approach)	Build/validate a diagnostic model to identify MASH patients in a high-risk population	Possible model parameters are all baseline clinical characteristics reported in the eCRF
	Explore genotypes related to MASH in different European countries	Genomic (GWAS) and proteomics analysis on collected blood samples
	Explore (non-invasive) metabolite biomarkers identifying MASH in patients at risk	Mass-spectrometry (MS) based metabolomic and lipidomic analyses and fluxomics analysis on collected blood samples, both targeted and untargeted approaches
	Prevalence of co-morbidities and associated therapies (especially for cardiovascular disease) in patients with MASH compared to those without, in high risk patient populations.	Prevalence of comorbidities in patients at- risk, patients diagnosed with MASLD/MASH
	Identify prognostic factors/biomarkers for complications in patients with MASLD and MASH at 5 years	Disease progression and liver-related and non-liver related complications
Evaluate Patient Reported Outcomes (PRO) from baseline throughout follow-up		PRO surveys at baseline and follow-up

Confirm high risk of liver fibrosis based on second FibroScan examination after lifestyle recommendations (in subset only)	Revisited FibroScan examination (Cap and LSM by VCTE) 12 weeks after first FibroScan and after lifestyle recommendations
To evaluate changes in CAP, LSM by VCTE and FAST scores over time	Repeated CAP, LSM by VCTE and FAST measurement over time

Data management

Clinical data will be captured using an electronic case report form (eCRF), only authorised staff at the CoE will be allowed to enter data into the eCRF. All eCRF data should be verifiable to a source at the CoE or accessible by the site staff, except if direct entry was allowed. Participants are identified by a code, only the local investigators will have the key. The investigator(s) will be responsible for ensuring eCRF data completeness and accuracy. Source data verification and data quality will be assessed by remote eCRF reviews, statistical checks and in-person monitor visits. Data from the eCRF and other sources (i.e., central analyses, Liver Health Management platform) will be encoded and stored in a central study database (Data Science Platform).

Statistical analysis

R will be used for statistical analysis, p-values < 0.05 (2-tailed) are considered statistically significant. By default, parametric testing will be employed. In case of not normally distributed variables, non-parametric tests will be employed. Before analysis, we will assess the degree and possible reasons for missingness and apply appropriate methods to handle missing data i.e., multiple imputation.

As the primary objective is the implementation of a patient care pathway (see table 4), primarily descriptive statistics will be used instead of hypothesis testing. Prevalence of MASLD, liver steatosis, fibrosis and (at-risk) MASH will be reported for each country. The prevalence will be compared between countries using one-way ANOVA (or Kruskal Wallis test). The overlap in numbers of patients at risk identified by the 2 NITs will be reported as well.

For the secondary objectives the following statistical methods will be used: diagnostic modelling using logistic regression and machine learning (ML)/artificial intelligence (AI) approaches; associations in GWAS and proteomics data will be analysed using logistic regression; associations between MASH diagnosis by histology and (non-invasive) metabolite (bio)markers will be analysed using logistic regression; prevalence of co-morbidities will be estimated in total group of patients at risk with/without MASLD, the difference between the groups will be analysed using one-way ANOVA (or Kruskal Wallis test); prognostic modelling using logistic regression and ML/AI approaches; mixed linear modelling will be implemented to assess changes in PRO over time; difference between 1st and 2nd LSM by VCTE measurements will be assessed using a paired t-test (or Wilcoxon signed rank test); mixed linear modelling will be implemented to assess the repeated measurements of CAP, LSM by VCTE and FAST over time.

Ethics and dissemination

The study will be conducted in compliance with this protocol, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), and applicable national and international regulatory requirements (including Declaration of Helsinki). The study initiation package is submitted at the local level. The study protocol has already been approved by local medical ethical committees in all 10 participating countries: Netherlands (MEC-U, A23.273/R22.057), Germany (Etik-Kommission Landesartzekammer Rheinland-Pfalz, 2022-16602), Spain (CEI de los Hospitales Universitarios Virgen Macarena y Virgen del Rocío, 1631-N-22), Belgium (Ethische Commissie Universitair Ziekenhuis Antwerpen, EDGE 002626) and Portugal (Comissao de Etica do CAML, 203/23) REST TO BE ADDED BEFORE PUBLICATION. Results will be made public and published in scientific, peer-reviewed, international journals and at international conferences.

Patient and Public Involvement

The draft protocol was reviewed and endorsed by Liver Patients International (LPI). The European Liver Patients Association (ELPA), the European Atherosclerosis Society (EAS) and the European Association for the Study of the Liver (EASL) have also endorsed the final project.

DISCUSSION

Although several factors are involved in the progression from MASLD to MASH (environmental, dietary, genetic), the exact mechanisms behind MASLD development and progression remain unknown [46]. The widely used estimate of 38% worldwide prevalence of MASLD is based on a meta-analysis of studies from 2016 to 2019 [6]. However, exact data about the prevalence in different European countries using accepted diagnostic tools like LSM by VCTE or the golden standard, liver biopsy, are still lacking. This study will provide prevalence estimates based on a large number of participants.

The patient care pathway used in this study is based on the 2021 and 2024 updates of the EASL clinical practice guidelines [23], [25]. In contrast to these guidelines, in the current protocol the two NITs (FIB-4 and FibroScan) will be performed in the same visit. Recently there has been debate about the accuracy of FIB-4 to (pre-)screen in the general population at primary care level [47], as the index was validated and developed in secondary and tertiary care. This issue was acknowledged by the EASL in 2021 and they already advised not to base diagnoses on one single NIT, but to use concordant NITs instead [23]. Therefore, in the current protocol it was decided to perform the FibroScan and blood sampling for FIB-4 at the same day for each patient. We can then compare the added value of FIB-4 versus FibroScan and their prognostic capacity at follow up within a population at risk identified at the primary care level and outpatient clinics, and thereby either validate the guidelines or propose adjustments.

In line with the EASL Clinical Practice Guidelines, structured programmes aimed at lifestyle changes towards healthy diet and habitual physical activity are advisable [16]. The current study only includes lifestyle recommendations to a subset of participants as this would be feasible within current clinical practice. The level of adherence to the Mediterranean diet will be evaluated throughout the study. The Mediterranean diet is thought to be beneficial [48], although evidence is still limited [49]. We also aim to build towards personalized lifestyle advice.

European experts have demonstrated that most countries in Europe are not yet ready to face the growing MASLD/MASH problem, because they lack guidelines, registries and multidisciplinary programs [30]. Therefore, initiatives such as this study aimed at increasing knowledge and awareness of MASLD and MASH among professionals are essential. In addition, this study aims to integrate all levels of clinical care, which is much needed for multidisciplinary management of MASLD.

Author contributions

DEG and MCC conceptualized the study. VDJ and MCC were the main authors of the manuscript and protocol. DEG, OHF and the members of the initial Julius Clinical-initiated Scientific Steering Committee (HCP, SF, CM, MRG, MET, AGH, LS, JMS, JWMM, AG, VR) and Independent Advisory Board (JW, MA) critically reviewed the initial protocol. TH contributed to the lipidomics/metabolomics section, JV and MD to the liver biopsy section. CFP, RB, GVD, LM, VR, DCS critically review the revised protocol. MCC is guarantor.

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Competing interests statement

JMS: Consultant: Akero, Alentis Therapeutics, Astra Zeneca, 89Bio, Boehringer Ingelheim, GSK, Ipsen, Inventiva Pharma, Madrigal, MSD, Northsea Therapeutics, Novartis, Novo Nordisk, Pfizer, Roche, Sanofi, Siemens Healthineers. Research Funding: Boehringer Ingelheim, Siemens Healthcare GmbH. Stock Options: AGED diagnostics, Hepta Bio. Speaker Honorarium: Gilead Sciences, Advanz, Echosens, MedPublico GmbH.

CM: Consulting fees: Julius Clinical, Echosens, Gilead.

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The other authors have no relevant financial or non-financial interests to disclose.

Provenance and peer review

The protocol was peer reviewed by the members of the initial Julius Clinical-initiated GRIPonMASH Scientific Steering Committee and Principal Investigators of the CoEs. The authors are thankful to Prof. J. Ordovas for providing critical feedback on the final version of this manuscript.

Data availability statement

Collected data will be available upon reasonable request. Only unidentified data are available to researchers who submit a methodologically sound proposal. Proposals may be submitted to the GRIPonMASH consortium for review. The consortium reserves the right to analyse data for development and intellectual property purposes prior to public disclosure.

REFERENCES

[1] M. E. Rinella et al., 'A multi-society Delphi consensus statement on new fatty liver disease nomenclature.', Hepatology, Jun. 2023, doi: 10.1097/HEP.0000000000000520.

[2] C. D. Byrne and G. Targher, 'NAFLD: a multisystem disease', J Hepatol, vol. 62, no. 1 Suppl, pp. S47–S64, 2015, doi: 10.1016/J.JHEP.2014.12.012.

[3] G. Targher, C. D. Byrne, A. Lonardo, et al., 'Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis', J Hepatol, vol. 65, no. 3, pp. 589–600, Sep. 2016, doi: 10.1016/J.JHEP.2016.05.013.

[4] M. Ekstedt et al., 'Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up', Hepatology, vol. 61, no. 5, pp. 1547–1554, May 2015, doi: 10.1002/HEP.27368.

[5] S. Driessen et al., 'Metabolic dysfunction associated steatotic liver disease and the heart', Hepatology, Dec. 2023, doi: 10.1097/HEP.0000000000000735.

[6] Z. M. Younossi, P. Golabi, J. M. Paik, et al., 'The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review.', Hepatology, Jan. 2023, doi: 10.1097/HEP.0000000000000004.

[7] J. Quek et al., 'Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis', Lancet Gastroenterol Hepatol, vol. 8, no. 1, pp. 20–30, Jan. 2023, doi: 10.1016/S2468-1253(22)00317-X.

[8] L. Henry, J. Paik, and Z. M. Younossi, 'Review article: the epidemiologic burden of non-alcoholic fatty liver disease across the world', Aliment Pharmacol Ther, vol. 56, no. 6, pp. 942–956, Sep. 2022, doi: 10.1111/APT.17158.

[9] Z. M. Younossi et al., 'The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis', J Hepatol, vol. 71, no. 4, pp. 793–801, Oct. 2019, doi: 10.1016/J.JHEP.2019.06.021.

[10] J. M. Schattenberg et al., 'Disease burden and economic impact of diagnosed non-alcoholic steatohepatitis in five European countries in 2018: A cost-of-illness analysis', Liver Int, vol. 41, no. 6, pp. 1227–1242, Jun. 2021, doi: 10.1111/LIV.14825.

[11] T. H. Karlsen et al., 'The EASL–Lancet Liver Commission: protecting the next generation of Europeans against liver disease complications and premature mortality', The Lancet, vol. 399, no. 10319, pp. 61–116, Jan. 2022, doi: 10.1016/S0140-6736(21)01701-3/ATTACHMENT/82513520-DAB3-430E-A196-83F57E6FC6C3/MMC1.PDF.

[12] E. E. Powell, V. W. S. Wong, and M. Rinella, 'Non-alcoholic fatty liver disease', Jun. 05, 2021, Elsevier B.V. doi: 10.1016/S0140-6736(20)32511-3.

[13] Z. M. Younossi, A. B. Koenig, D. Abdelatif et al., 'Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes', Hepatology, vol. 64, no. 1, pp. 73–84, Jul. 2016, doi: 10.1002/HEP.28431/SUPPINFO.

[14] J. F. Dufour et al., 'The global epidemiology of nonalcoholic steatohepatitis (NASH) and associated risk factors–A targeted literature review', Endocrine and Metabolic Science, vol. 3, p. 100089, Jun. 2021, doi: 10.1016/J.ENDMTS.2021.100089.

[15] A. J. Sanyal et al., 'Prospective Study of Outcomes in Adults with Nonalcoholic Fatty Liver Disease', N Engl J Med, vol. 385, no. 17, pp. 1559–1569, Oct. 2021, doi: 10.1056/NEJMOA2029349.

[16] G. Marchesini et al., 'EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease', J Hepatol, vol. 64, no. 6, pp. 1388–1402, Jun. 2016, doi: 10.1016/J.JHEP.2015.11.004.

[17] F. E. Mózes et al., 'Performance of non-invasive tests and histology for the prediction of clinical outcomes in patients with non-alcoholic fatty liver disease: an individual participant data meta-analysis', Lancet Gastroenterol Hepatol, vol. 8, no. 8, pp. 704–713, Aug. 2023, doi: 10.1016/S2468-1253(23)00141-3.

[18] M. Noureddin et al., 'NASH Leading Cause of Liver Transplant in Women: Updated Analysis of Indications For Liver Transplant and Ethnic and Gender Variances', Am J Gastroenterol, vol. 113, no. 11, pp. 1649–1659, Nov. 2018, doi: 10.1038/S41395-018-0088-6.

[19] V. Ratziu, 'Non-pharmacological interventions in non-alcoholic fatty liver disease patients', Liver International, vol. 37, pp. 90–96, Jan. 2017, doi: 10.1111/LIV.13311.

[20] M. Ren, X. Zhou, L. Lv, et al., 'Endoscopic Bariatric and Metabolic Therapies for Liver Disease: Mechanisms, Benefits, and Associated Risks', J Clin Transl Hepatol, vol. 10, no. 5, p. 986, Oct. 2022, doi: 10.14218/JCTH.2021.00448.

[21] S. A. Harrison et al., 'A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis', N Engl J Med, vol. 390, no. 6, pp. 497–509, Feb. 2024, doi: 10.1056/NEJMOA2309000.

[22] F. Kanwal et al., 'Clinical Care Pathway for the Risk Stratification and Management of Patients With Nonalcoholic Fatty Liver Disease', Gastroenterology, vol. 161, no. 5, pp. 1657–1669, Nov. 2021, doi: 10.1053/J.GASTRO.2021.07.049.

[23] A. Berzigotti et al., 'EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update', J Hepatol, vol. 75, no. 3, pp. 659–689, Sep. 2021, doi: 10.1016/J.JHEP.2021.05.025.

[24] A. Srivastava et al., 'Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease', J Hepatol, vol. 71, no. 2, pp. 371–378, Aug. 2019, doi: 10.1016/J.JHEP.2019.03.033.

[25] Association for the Study of the Liver et al., 'EASL–EASD–EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD)', J Hepatol, vol. 0, no. 0, Jun. 2024, doi: 10.1016/J.JHEP.2024.04.031.

[26] M. E. Rinella et al., 'AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease.', Hepatology, Feb. 2023, doi: 10.1097/HEP.0000000000000323.

[27] K. Cusi et al., 'American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD)', Endocrine Practice, vol. 28, no. 5, pp. 528–562, May 2022, doi: 10.1016/J.EPRAC.2022.03.010.

[28] P. Ferenci et al., 'World Gastroenterology Organisation Global Guidelines Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis', 2014. [Online]. Available: www.jcge.com

[29] Q. M. Anstee et al., 'Real-world management of non-alcoholic steatohepatitis differs from clinical practice guideline recommendations and across regions', JHEP Reports, vol. 4, no. 1, Jan. 2022, doi: 10.1016/j.jhepr.2021.100411.

[30] J. v. Lazarus et al., 'European "NAFLD Preparedness Index" — Is Europe ready to meet the challenge of fatty liver disease?', JHEP Reports, vol. 3, no. 2, p. 100234, Apr. 2021, doi: 10.1016/J.JHEPR.2021.100234.

[31] J. M. Schattenberg, Q. M. Anstee, C. Caussy, et al., 'Differences between current clinical guidelines for screening, diagnosis and management of nonalcoholic fatty liver disease and real-world practice: a targeted literature review', 2021, Taylor and Francis Ltd. doi: 10.1080/17474124.2021.1974295.

[32] S. Driessen et al., 'A global survey of health care workers' awareness of non-alcoholic fatty liver disease: The AwareNASH survey', United European Gastroenterol J, 2023, doi: 10.1002/UEG2.12445.

[33] Y. Vali et al., 'Biomarkers for staging fibrosis and non-alcoholic steatohepatitis in non-alcoholic fatty liver disease (the LITMUS project): a comparative diagnostic accuracy study', Lancet Gastroenterol Hepatol, vol. 8, no. 8, pp. 714–725, Aug. 2023, doi: 10.1016/S2468-1253(23)00017-1.

[34] P. J. Eddowes et al., 'Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease', Gastroenterology, vol. 156, no. 6, pp. 1717–1730, May 2019, doi: 10.1053/J.GASTRO.2019.01.042.

[35] V. W. S. Wong et al., 'Unified interpretation of liver stiffness measurement by M and XL probes in non-alcoholic fatty liver disease', Gut, vol. 68, no. 11, pp. 2057–2064, 2019, doi: 10.1136/GUTJNL-2018-317334.

[36] W. Theel et al., 'Evaluation of nonalcoholic fatty liver disease (NAFLD) in severe obesity using noninvasive tests and imaging techniques', Obesity Reviews, vol. 23, no. 8, p. e13481, Aug. 2022, doi: 10.1111/OBR.13481.

[37] J. v Lazarus et al., 'Real-world evidence on non-invasive tests and associated cut-offs used to assess fibrosis in routine clinical practice', JHEP Reports, vol. 0, no. 0, p. 100596, Sep. 2022, doi: 10.1016/J.JHEPR.2022.100596.

[38] E. J. Grobbee et al., 'Improvement of non-invasive tests of liver steatosis and fibrosis as indicators for non-alcoholic fatty liver disease in type 2 diabetes mellitus patients with elevated cardiovascular risk profile using the PPAR-α/γ agonist aleglitazar', PLoS One, vol. 17, no. 11, p. e0277706, Nov. 2022, doi: 10.1371/JOURNAL.PONE.0277706.

[39] P. N. Newsome et al., 'FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study', Lancet Gastroenterol Hepatol, vol. 5, no. 4, pp. 362–373, Apr. 2020, doi: 10.1016/S2468-1253(19)30383-8.

[40] A. C. Sheka, O. Adeyi, J. Thompson, et al., 'Nonalcoholic Steatohepatitis: A Review', JAMA, vol. 323, no. 12, pp. 1175–1183, Mar. 2020, doi: 10.1001/JAMA.2020.2298.

[41] F. Nascimbeni et al., 'Clinical validation of the FLIP algorithm and the SAF score in patients with non-alcoholic fatty liver disease', J Hepatol, vol. 72, no. 5, pp. 828–838, May 2020, doi: 10.1016/J.JHEP.2019.12.008.

[42] M. A. Martínez-González et al., 'A 14-Item Mediterranean Diet Assessment Tool and Obesity Indexes among High-Risk Subjects: The PREDIMED Trial', PLoS One, vol. 7, no. 8, Aug. 2012, doi: 10.1371/JOURNAL.PONE.0043134.

[43] C. L. Craig et al., 'International physical activity questionnaire: 12-country reliability and validity', Med Sci Sports Exerc, vol. 35, no. 8, pp. 1381–1395, Aug. 2003, doi: 10.1249/01.MSS.0000078924.61453.FB.

[44] C. Sancho-Domingo, J. L. Carballo, A. Coloma-Carmona et al., 'Brief version of the Pittsburgh Sleep Quality Index (B-PSQI) and measurement invariance across gender and age in a population-based sample', Psychol Assess, vol. 33, no. 2, pp. 111–121, Feb. 2021, doi: 10.1037/PAS0000959.

[45] S. M. Francque et al., 'Non-alcoholic fatty liver disease: A patient guideline'.

[46] Q. M. Anstee et al., 'Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort☆', J Hepatol, vol. 73, no. 3, pp. 505–515, Sep. 2020, doi: 10.1016/J.JHEP.2020.04.003.

[47] I. Graupera et al., 'Low Accuracy of FIB-4 and NAFLD Fibrosis Scores for Screening for Liver Fibrosis in the Population', Clin Gastroenterol Hepatol, vol. 20, no. 11, pp. 2567-2576.e6, Nov. 2022, doi: 10.1016/J.CGH.2021.12.034.

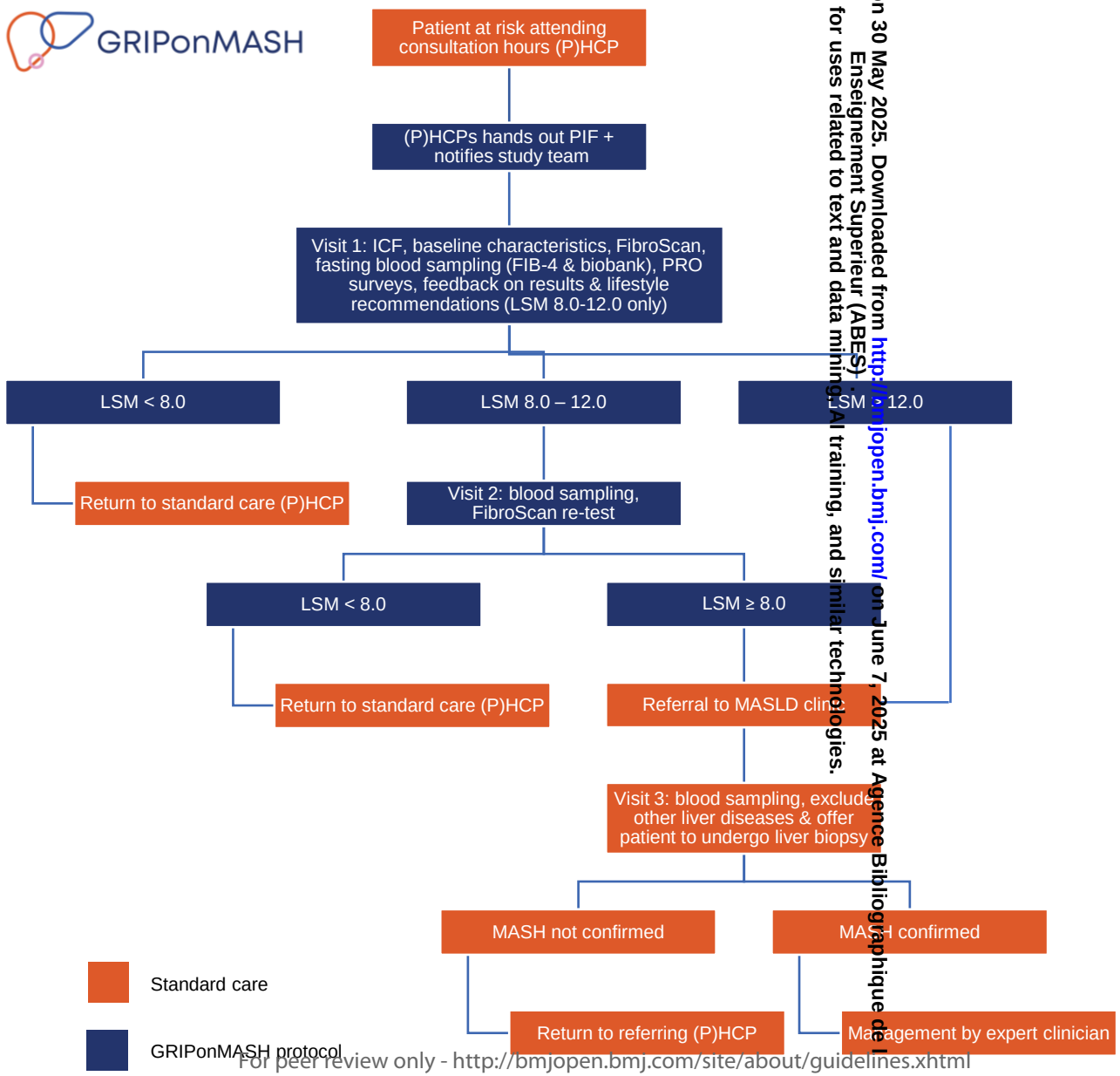
[48] Y. Gepner et al., 'The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content', J Hepatol, vol. 71, no. 2, pp. 379–388, Aug. 2019, doi: 10.1016/J.JHEP.2019.04.013.

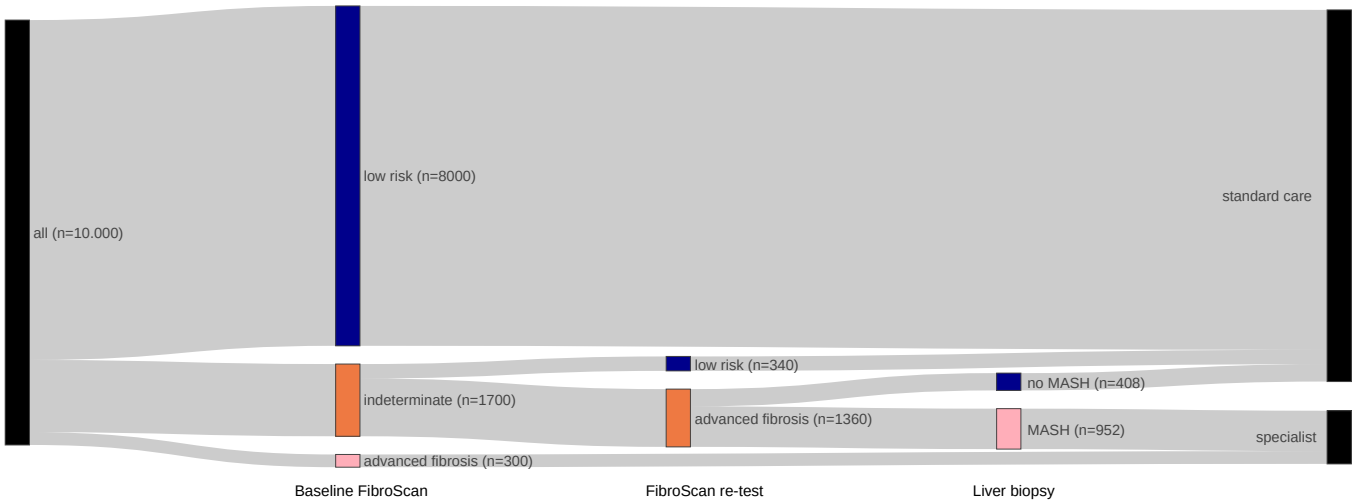
[49] M. C. P. Torres et al., 'Mediterranean Diet and NAFLD: What We Know and Questions That Still Need to Be Answered.', Nutrients, vol. 11, no. 12, pp. E2971–E2971, Dec. 2019, doi: 10.3390/NU11122971.

Figure legends

Figure 1: Overview of study design and patient flow. Abbreviations used: (P)HCP = (primary) health care provider, PIF = patient information folder, ICF = informed consent form, FIB-4 = Fibrosis-4 index, LSM = liver stiffness measurement by VCTE.

Figure 2: Expected patient flow through patient care pathway. Based on estimates available in literature, in the total at risk population of 10.000 participants, we expect 80% to score LSM < 8 kPa (low risk), 17% is expected to score LSM 8 – 12 kPa (indeterminate) and 3% LSM > 12 kPa (advanced fibrosis) upon screening with FibroScan. We assume at least 80% will have LSM ≥ 8 kPa at the re-test. Of those who will be offered liver biopsy, we expect that for 70% MASH diagnosis will be confirmed upon biopsy. In theory this leads to approximately 950 biopsy confirmed diagnoses of MASH. However, considering reluctance for invasive testing and loss to follow-up, we expect to identify approximately 500 confirmed MASH patients in this study, and thus on average 50 per country.





SUPPLEMENTAL MATERIAL I

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Subject information for participation in medical research

Screening of patients at risk for liver problems due to fat buildup in the liver (GRIPonMASH)

"Global Research Initiative for Patients screening on MASH"

GRIPonMASH

Julius Clinical

Introduction

Dear Sir/Madam,

With this letter, we would like to ask you to take part in a medical study. Participation is voluntary. You have received this letter because your health care provider found you are at risk of developing liver problems due to fat buildup in your liver and it is possible to be screened for this.

You can read about the medical study in this information sheet. We will explain what it means for you and what the pros and cons of participation are. It is a lot of information. Can you please read the information carefully and decide if you want to take part? If you want to take part, complete the form in Appendix E.

Feel free to ask your questions
You can take your decision based on the information in this information sheet. We also suggest that you do this:

- Ask questions to the health care provider who gave you this information.
- Talk to your partner, family or friends about this study.
- Read the information on: [refer to government information website, if applicable].

1. General information

Julius Clinical has set up this study together with commercial and non-commercial research partners in Europe. This is called a research consortium. Julius Clinical is a research institute located in Zeist, the Netherlands. Below, we always call Julius Clinical the 'sponsor'. Investigators, these can be doctors, research nurses or other hospital staff, carry out the research in different hospitals in collaboration with associated general practitioners. In [country] these are [names of hospitals]. A central Julius Clinical research team oversees the entire study.

This study needs 10.000 subjects from 10 different countries. In [specific county], it is expected that 1000 patients will take part. The Medical Ethics Review Committee [X] has approved this study.

You will have at least one week to decide if you want to take part in the study. After this week you will be approached by the investigator and you can let him/her know your decision.

2. What is the purpose of the study?

This study screens 10.000 patients in 10 countries with an increased risk of liver problems due to fat buildup in the y liver. This way we learn more about how often these liver problems occur and how they can be detected earlier. With the screening program we hope to improve the collaboration between general practitioners and specialized clinics/hospitals by introducing a so-called 'patient care pathway'. This care path has been established by international scientific associations. The current study is investigating whether less invasive methods to detect liver problems (blood tests and 'FibroScan') work just as well as liver biopsy (where a small sample is taken form the liver). We are also trying to find other ways to detect and predict liver problems.

3. What is the background of the study?

More and more people suffer from overweight, obesity and associated diseases like type 2 diabetes mellitus. This is a growing healthcare concern, and it is for example related to an increased risk of cardiovascular diseases. It is also related to metabolic dysfunction-associated steatotic liver disease (MASLD). MASLD is a long-lasting liver condition caused by fat buildup in the liver. MASLD often interferes with the proper functioning of the liver. MASLD can lead to inflammation of the liver (called metabolic dysfunction-associated steatohepatitis; MASH) and scarring of the liver (called fibrosis). Severe MASLD can ultimately cause liver failure (cirrhosis) and can even lead to liver cancer (hepatocellular carcinoma), although the chance of having these severe complications is very small.

The risk of developing MASLD is higher if you have obesity, diabetes, arterial hypertension and 'metabolic syndrome'. It is not yet known exactly how many people in Europe have MASLD. In the early stages MASLD is reversible. That is why it is so important to detect patients with MASLD at an early stage and to start in time with available treatments , like adjusting your lifestyle and in some cases bariatric surgery. Lifestyle changes can for example be, what and how much you eat, and how much you exercise.

4. What happens during the study?

How long will the study take?
Are you taking part in the study? The screening program will be completed within 8 months after enrolling in the study in the hospital. In the third and fifth year after the start, you will be asked again for a short check in the hospital. So the total study duration is 5 years.

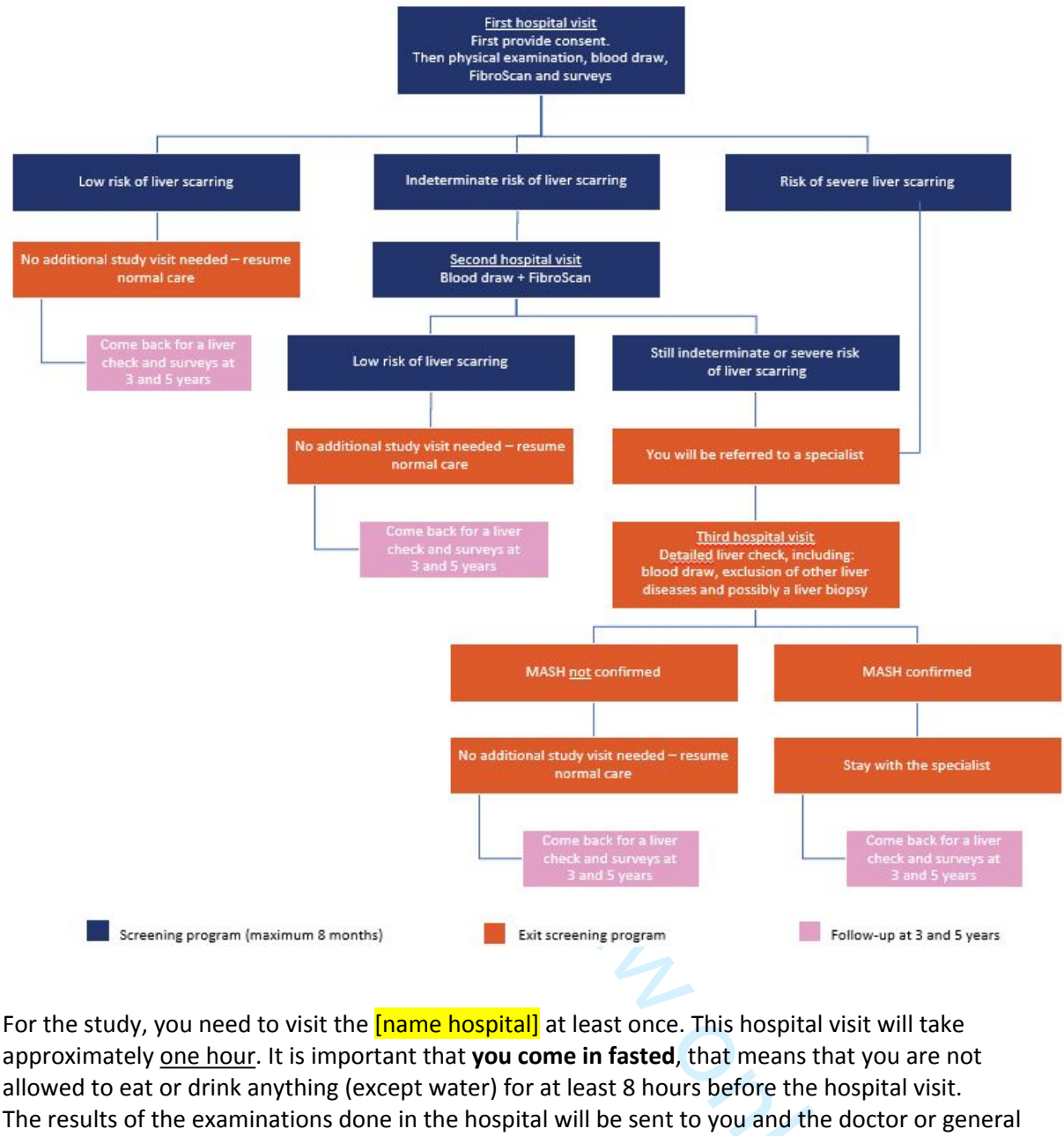
Step 1: are you eligible to take part? This step will take around 10 minutes
First, we want to know if you are eligible to take part. That is the reason that the investigator or general practitioner will do some checks:

- Your medical history.
  - You are only eligible for the screening if you have an increased risk of developing MASLD. In this study defined as: people with obesity, type 2 diabetes, high blood pressure and metabolic syndrome.
  - You cannot have any other liver diseases.
- Excessive alcohol consumption can also damage the liver, so the investigator will ask about your average alcohol consumption.
  - You cannot participate if you consume more than 2-3 units of alcohol per day.

Please note: it is possible that due to other reasons you are not eligible for this study. The investigator will tell you more about this.

Step 2: study and measurements
If you are eligible to participate in the study, you are invited to come to the [name hospital] for further investigation. In the hospital we will first confirm if you are eligible to take part in the study. Then we will check if you have fat buildup or scar tissue on your liver using a kind of ultrasound machine (FibroScan). The outcome of that test determines the further steps within the screening program.

An overview of the screening program (dark blue) and corresponding follow-up checks in the third and fifth year are shown in the figure below:



For the study, you need to visit the [name hospital] at least once. This hospital visit will take approximately one hour. It is important that **you come in fasted**, that means that you are not allowed to eat or drink anything (except water) for at least 8 hours before the hospital visit. The results of the examinations done in the hospital will be sent to you and the doctor or general practitioner who referred you to the study.

We will carry out these checks during the first visit to the hospital:

- Short physical examination. For example, the examiner may measure your blood pressure, weight, length and waist circumference.
- Blood draw. For this, the investigator takes 8 tubes of blood. In total, we will collect approximately 47,5 ml of blood from you. This amount does not cause any problems in adults. For comparison: if you give blood at the blood bank, you will give 500 ml of blood at a time. With the blood test, we measure:
    - Your liver status values
    - Other values related to your health, such as cardiovascular risk factors.
    - If your genes in your DNA are related to a higher risk of MASLD.
    - If there are compounds (such as fats or proteins) in your blood that can predict if you have MASLD or not.
- FibroScan. This is a kind of liver ultrasound that can estimate the amount of fat buildup and scarring of the liver.

- Questionnaires about your diet and lifestyle.
- If the FibroScan provides an indeterminate result, lifestyle recommendations will be provided.
- You may be invited for an interview about your diet, this will extend the visit by 30 minutes. The interview will be conducted by a dietitian or a trained research assistant.

Depending on the results from the first visit, the next visits are scheduled or no additional visits are required:

- *Low risk of liver scarring*: no additional visit needed.
- *Indeterminate risk of liver scarring*: You will be asked to visit the hospital again after 12 weeks for a second FibroScan. We will collect 3 tubes of blood (total approximately 8,5 ml). This will take around 30 minutes.
    - If your liver status improved, no additional visit is needed.
    - If your liver status did not improve, you will be referred to a specialist who will check your liver in more detail. A liver biopsy is performed to be able to definitively diagnose if you have MASH or not. You will be asked to visit the hospital again, and again you need to be fasted. This visit will take around 4 hours as you will have to lay down for a while after the biopsy. We will collect 3 tubes of blood (total approximately 16,5 ml).
- *Severe liver scarring*: you will immediately be referred to a specialist who will check your liver in more detail, according to standard care. We will collect 3 tubes of blood (total approximately 16,5 ml).

Step 3: follow-up check
Three and five years after your first visit, you will be asked to visit the hospital again. At both visits a short physical examination is done. We will collect 3 tubes of blood (total approximately 16,5 ml) and you will be asked to complete the diet and lifestyle questionnaires. At the three year visit we will also do another FibroScan. The results of the FibroScan will again be shared with you and the doctor or general practitioner who referred you to the study.

Appendix C has a list of the measurements we carry out during each visit.

What is the difference with standard care?
This study is an addition to your regular care. The screening program and follow-up checks can be done next to your regular care.

5. What agreements do we make with you?

We want the study to go well. That is why we want to make the following agreements with you:

- You go to every appointment.
- You should contact the investigator in these situations:
    - You are hospitalised or get treatment in a hospital.
    - You suddenly have problems with your health.
    - You no longer want to take part in the study.
    - Your telephone number, address or email address changes.

The next two paragraphs are only applicable for female participants.

Is it OK for you to get pregnant during the study?
This study cannot have any consequences for an unborn child, however we do not know if the

FibroScan does provide a reliable result during pregnancy. Therefore, women who are pregnant cannot participate in this study.

Pregnant after all?
If you do become pregnant during the study, then let investigator know. In the event of a pregnancy, where a FibroScan was still scheduled, you should postpone participation in the study until after the birth in consultation with the investigator.

6. What are the pros and cons if you take part in the study?

Taking part in the study can have pros and cons. We will list them below. Think about this carefully and talk to other people about it.

If you follow the screening program, it will become clear whether you have MASLD or not. If you participate in this research, it does not mean that MASLD will be cured or that you will suffer less from the disease, but your participation will help the investigators to gain more insight into the timely detection of MASLD. You will also help the investigator to gain more insight into the occurrence of MASLD in different countries.

Taking part in the study can have these cons:

- There may be some discomfort from the measurements during the study. For example: taking a blood sample can be a little painful. Or you could get a bruise as a result.
- Taking part in the study will cost you extra time.
- You have to comply with the study agreements.

What are the possible discomforts you may experience with checks or measurements during the study?
Blood sampling is a regular medical procedure with low risks, although in rare cases you may develop bruising after the blood sampling. The FibroScan is a kind of ultrasound and thus has no risks.

In a selected group of patients (see Figure 1) a liver biopsy will be performed. With the collection of liver tissue, there is a risk of bleeding. This bleeding will be cared for immediately.

When you are referred to a specialist to check your liver in more detail, , the specialist will ask for your permission again as these checks are part of standard care. If you participate in the study you give the investigator permission to collect the results of these checks. In case of a liver biopsyyou give permission to collect the results and a bit of your liver tissue.

Possibility of accidental discoveries
It is possible that an accidental discovery is made during the study (for example during the FibroScan, with the blood testing or genetic examination) that is not directly related to the research, but does concern your health. If this happens, your own doctor or specialist will discuss with you what needs to happen next.

You do not wish to participate in the study?
It is up to you to decide if you wish to participate in the study. Do you not wish to participate? You will not be screened for the presence of MASLD and you will continue your health care visits as usual.

7. When does the study end?

The investigator will let you know if there is any new information about the study that is important to you. The investigator will then ask you if you want to continue to take part.

In these situations, the study will stop for you:

- All checks according to the schedule are finished.
- You have become pregnant (in this case the study will be postponed until after the birth).
- You want to stop participating in the study yourself. You can stop at any time. Report this to the investigator immediately. You do not have to explain why you want to stop. You will then exit the screening program and resume your usual health care visits. The investigator will no longer collect information from the moment you indicate that you want to stop participating in the study.
- The investigator thinks it is better for you to stop. The investigator will still invite you for a follow-up check.
- One of the following authorities decides that the study should stop:
    - Julius Clinical
    - the government, or
    - the Medical Ethics Review Committee assessing the study

What happens if you stop participating in the study?
The investigators use the data and body material (the blood samples, and in some cases liver samples) that have been collected up to the moment that you decide to stop participating in the study. If you wish, we will destroy the collected body material. Please let the investigator know. Investigators will always be able to use the data that was collected between the moment you started the study and the moment that you indicated that you want to stop participating in the study.

The entire study ends when all the participants have finished.

8. What happens after the study has ended?

The first analysis is planned within 3 years after the start of the study. You will then receive a report about the most important results of the study.

9. What will be done with your data and body material?

Are you taking part in the study? Then you also give your consent to collect, use and store your data and body material.

What data do we store?
We store these data:

- your gender
- your ethnicity
- your date of birth (month and year only)
- information about your health
- (medical) information that we collect during the study
- Your name
- Your e-mail address
- Your phone number

- Your address
- Results of tests done during the study

What body material do we store?
We store the tubes of blood, and in some case pieces of liver tissue (biopsies). Your DNA can be analysed from the blood and tissue, or other compounds (such as fat and proteins) can be measured. This is further explained in Appendix D.

Why do we collect, use and store your data and body material?
We collect, use and store your data and your body material to answer the questions of this studyand to be able to publish the results.

What do we do with your data?
In this study we work together with commercial and non-commercial partners, which form a research consortium together. The collected data and test results may be shared with these partners to answer the research questions. Also your data will be added to a healthcare platform.

How do we protect your privacy?
To protect your privacy, we give a code to your data and your body material. We only put this code on your data and body material. We keep the key to the code in a safe place in the hospital. When we process your data and body material, we always use only that code. Even in reports and publications about the study, nobody will be able to see that it was about you.

Who can see your data?
Some people can see your name and other personal information without a code. Your referring health care provider and the staff at the hospital will know who you are. The hospital staff executes all physical measurements and collects the data and body material.

The body material is sent to the central storage facility at UMC Utrecht in The Netherlands. The body material will only be referred to by the code, so employees of the central storage facility only know the code. The hospital staff enters the collected data into the central research database. Only coded information is entered, so the central research team at Julius Clinical or other consortium partners only know the code.

Other people who are allowed to see your information without a code, are people checking whether the investigators are carrying out the study properly and reliably. These persons can access your data:

- An auditor and/or monitor who works for the sponsor or is hired by the sponsor.
- National and international supervisory authorities.

These people will keep your information confidential. We ask you to give permission for this access.

For how long do we store your data and body material?
We store your data at the hospital for 15 years. And for 15 years with the sponsor. We store your body materials at the central storage facility at the Utrecht UMC (UMCU Biobank). They will be stored for a maximum of 15 years in order to be able to make new assessments related to this study or in the course of this study. If no longer needed, we will destroy your body material.

Can we use your data and body material for other research?
Your data and your remaining body material may also be important after this study for other medical research on MASLD and related liver problems, such as improving detection methods and developing possible treatment options. For this purpose, remaining body material will be stored at the central

storage facility at the UMC Utrecht for 15 years. Your data will be stored centrally for 15 years. Please indicate in the consent form whether you agree with this. Do you not want to give your consent for this? Then you can still take part in this study. You will enter the same screening program. When a request is made for the use of your data and/ or for the use of your remaining body material, the sponsor will first determine if the request is in line with your consent. If so, the sponsor will request the central storage facility to release the requested remaining body material and/ or will provide the requester with your data. If the other research is of a commercial nature, you as participant will not take share in any possible commercial gains and will not gain a right to the investigational product or medicine.

What will be done with your body material?
Blood samples will be divided in three groups. The first group will be analysed in the hospital. The second and third groups will be shipped to the central storage facility at the UMC Utrecht. The second group of samples will be shipped from the central storage facility to different laboratories and commercial and non-commercial partners in Europe to be analysed. The third group of samples will stay in the storage facility and will be used if additional measurements are needed to answer the research questions or, if you agreed, for other related research.

Liver tissue samples will be sent to the central storage facility at the UMC Utrecht or central analysis directly. Analyses are done at a central laboratory or at commercial and/or non-commercial partners in Europe.

Remaining body material will be stored in the central storage facility and will be used if further or other analysis is needed to answer the research questions or, if you agreed, will be used for other related research.

What happens if there are accidental discoveries?
It is possible that during the study we discover something that is important to your health. In that case, the investigator will contact your referring health care provider. You will then discuss what needs to be done with them.. By signing the form (Appendix E), you give consent to inform your referring health care provider.

Can you take back your consent for the use of your data?
You can withdraw your consent for the use of your data at any time. This applies both to the use in this study and to the use in other medical research. But please note: if you withdraw your consent, and the investigators have already collected data for research, they are still allowed to use this information. The investigators will destroy your body material after you withdraw your consent if you ask them to do so. But if assessments with your body material have been carried out, the investigator can continue to use the results.

Do you want to know more about your privacy?

- Do you want to know more about your rights when processing personal data? Visit [refer to government website if possible].
- Do you have questions about your rights? Or do you have a complaint about the processing of your personal data? Please contact the person who is responsible for processing your personal data. For the present, this is:
    - [name hospital]. See Appendix A for contact details and website.
- If you have any complaints about the processing of your personal data, we recommend that you first discuss them with the research team. You can also contact the Data Protection Officer of [the hospital]. Or you can submit a complaint to the [local Data Protection Authority].

Where can you find more information about the study?
You can find more information about the study on the following website: https://griponmash.eu/
After the study, the website may show a summary of the results of this study.

10. Will you receive compensation if you participate in the study?

Participating in the screening program will not cost you anything. For participating in this study, you will receive an [site-specific: i.e., expense allowance of a maximum of €10,- per visit to the hospital, as a contribution towards travel and parking costs]. If you stop before you have visited the hospital, you will not be reimbursed for expenses.

11. Are you insured during the study?

Insurance has been taken out for everyone who takes part in this study. The insurance pays for damage caused by the study. But not for all damage. You can find more information about this insurance and any exceptions in Appendix B. It also says who you can report damage to.

12. We will inform your general practitioner

The investigator will send your referring health care provider an email to let them know that you are taking part in the study.

As part of the screening program the referring health care provider and research staff of the hospital will exchange information. This following information will be exchanged:

- Results of tests and/or diagnosis or medication use related to the conditions required to join the study.
- The result of the FibroScan (including CAP, LSM, FIB-4 and FAST) will be sent from the hospital to your referring health care provider.
- Results of urine tests related to liver diseases of the past six months (if available) will be sent from your referring health care provider to the research staff at the hospital.

By signing the form (Appendix E), you give consent to the exchange of information as described above.

13. Do you have any questions?

You can ask questions about the study to the research staff at the hospital.

Do you have a complaint? Discuss it with the doctor who is treating you. If you prefer not to do so, please visit [complaints officer/complaints committee of your hospital]. Appendix A tells you where to find this.

14. How do you give consent for the study?

You can first think carefully about this study. Then you tell the investigator if you understand the information and if you want to take part or not. If you want to take part, fill in the consent form that you can find with this information sheet. You and the investigator will both get a signed version of this consent form.

Thank you for your attention.

15. Appendices to this information

A. Contact details <to be adjusted per participating centre>

B. Information about the insurance

C. Schedule of study measurements

D. More information about measurements

E. Consent form

F. Withdrawal from prior consent

A. Contact details hospital and Julius Clinical

If you have any further questions about this study, please contact the research staff at your hospital.

Study centers [text below to be completed for each center]

Center X
Local investigator: [for principal investigator of centre: name, contact details (including phone number) and accessibility]

< if applicable>
[Study nurse/study doctor/nurse specialist]:

Complaints: [service or person with contact details and accessibility]

Data Protection Officer of the institution:

For more information about your rights: [Contact details [including website] of the person(s) responsible for processing personal data]:

<if applicable, to be supplemented with, for example, a coordinating investigator and/or an emergency number/24-hour availability>

Central investigation team at Julius Clinical
Coordinating principal investigators: Assoc. Prof. M. Castro Cabezas and Prof. D.E. Grobbee
Address: Julius Clinical, Broederplein 41-43, 3703 CD Zeist, The Netherlands
Website: https://www.juliusclinical.com/contact/
E: griponmash@juliusclinical.com

B. Information about the insurance

To be added

GRIPonMASH

Julius Clinical

C. Overview of study measurements

Depending on the outcome of visit 1, either no extra visits are needed, or you will be invited for visit 2 and then possibly visit 3.

	Screening program					Long-term follow up	
Who needs to go to this visit?	All patients		Only if indeterminate risk at 1st FibroScan	Only if risk of severe liver scarring at 1st FibroScan	Only if risk of severe liver scarring at 2nd FibroScan	All patients	
Name of visit	Pre-screening	Visit 1	Visit 2	Visit 3	Visit 4	Follow-up 1	Follow-up 2
Time (from first hospital visit)	- 4 weeks	0	12 weeks	16 weeks	30 weeks	3 years	5 years
Location	GP or hospital	Hospital	Hospital	Hospital	Hospital	Hospital	Hospital
Inclusion/exclusion criteria	x	x					
Demographics (birth date, gender, ethnicity)		x					
Medical history		x					
Medication use		x				x	x
Blood pressure (mm Hg)		x	x			x	x
Height (m)		x					
Weight (kg)		x	x			x	x
Waist circumference (cm)		x	x			x	x
Request recent urine laboratory measurements from health care provider		x					
Questionnaires diet and lifestyle		x				x	x
FibroScan		x	x			x	x
Blood draw		x	x	x	x	x	x
Feedback on FibroScan results		x				x	x
Diet and lifestyle review and lifestyle recommendations (only if indeterminate risk		x					
Optional: interview about diet		x		x	x		

D. More information about study measurements

Genetic and DNA research
We will analyse your genes in your DNA from the blood that will be collected. These genes may be related to the development and/or cure of MASLD. We expect that even more genes will be discovered that play a role in the coming years, so we would like to keep your blood sample for 15 years for further research.

Metabolomic, lipidomic, proteomic and fluxomic research
We will also look at different compounds (such as fats and proteins) that can be found in the blood. For example, we are interested to see if these compounds can predict if you have MASLD or not. Again, we expect that more will be possible in the coming years, and we would like to keep your blood for further research.

FibroScan
The FibroScan is a kind of ultrasound device that works on the basis of pressure waves. Please find a picture of the FibroScan device below. There is no need to take off your clothes for the measurement, only the abdomen must be exposed. The location of the liver is determined and some gel will be applied. For the measurement, the ultrasound probe will be held above the liver and will emit sound waves. The examination is not painful and takes around 10 minutes.

It is important that you arrive at the appointment fasted, meaning you are not allowed to eat or drink anything for some time before the appointment. You will receive more information about the precise rules from your local researchers.

Example of a FibroScan measurement

Liver biopsy (optional)
First, the skin above your liver will be numbed. The doctor will then make a small incision in the skin and take a small piece of tissue from the liver through a hollow needle. A liver biopsy is a safe test. In a small number of cases, punctures in the liver may cause bleeding or post-bleeding. That is why you will be asked to lie down for at least 3 hours after the examination so that the doctors can monitor this. You must also arrive fasted at this appointment. We expect that for approximately 20% of the participants a liver biopsy will be advised to determine what liver disease you have and how severe it is.

GRIPonMASH Julius Clinical

E. Consent form(s)

Belonging to: *Screening of patients at risk for liver problems due to fat buildup in the liver (GRIPonMASH)*

- I have read the information sheet. I was able to ask questions. My questions have been answered well enough. I had enough time to decide if I wanted to take part.
- I know that taking part is voluntary. I also know that at any time I can decide not to take part in the study. Or to stop taking part. I do not have to explain why.
- I give the investigator consent to inform my referring health care provider that I am taking part in this study.
- I give consent to request information from my referring health care provider about the tests and/or diagnosis or medication use related to conditions to participate in this study and results of urine measurements of the past six months (if available).
- I give consent to give my referring health care provider information about accidental discoveries made during the study that are important for my health.
- I give consent to collect, store, ship and use the results and body material that were obtained during procedures, carried out under standard of care as mentioned in the information letter.
- I give consent to collect, store, ship and use my data and body material. The investigators only do this to answer the question of this study.
- I know that some people will be able to see all of my data to review the study. These people are mentioned in this information sheet. I give consent to let them see my data for this review.
- I know that if I become pregnant, the study appointments are postponed until after the birth.

- Please tick yes or no in the table below.

I give consent to store my data to use for other research, as stated in the information sheet. The data will be stored for this purpose for another 15 years.	Yes ☐	No☐
I give consent to have my (remaining) body material stored and shipped for use in other research, as stated in the information sheet. The body material is stored for this purpose for another 15 years.	Yes ☐	No☐
I give consent to have my DNA (from blood and tissue) stored for use in other research, as stated in the information letter. The DNA is then stored for another 15 years.	Yes ☐	No☐
I give consent to ask me after this study if I want to participate in a follow-up study or drug development research.	Yes ☐	No☐

- I want to take part in this study.

My name is (subject): ……
Signature: …… Date : __/__/__

I declare that I have fully informed this subject about the study mentioned.

If any information becomes known during the study that could influence the subject's consent, I will let this subject know in good time.

Investigator name (or their representative): ……
Signature:…… Date: __/__/__

The study subject will receive a complete information sheet, together with a signed version of the consent form.

GRIPonMASH

Julius Clinical

F. Withdrawal Form for Prior Consent

Belonging to: *Screening of patients at risk for liver problems due to fat buildup in the liver (GRIPon MASH)*

I hereby give notice that I withdraw my participation in GRIPonMASH. This means that no new body material may be taken from me and no more medical data may be collected for GRIPonMASH.

I understand that body material that has been collected from me and has already been processed in the investigation, cannot be recovered or destroyed. Furthermore, I am aware that the medical records used in the study are not recovered or destroyed. This body material and medical data remain encoded and available to the person conducting the investigation.

With regard to the my body materials still stored for GRIPonMASH, I declare that my body material:

O may still be used according to the consent form I previously signed,

O must be destroyed.

My name is (subject): ……

Signature: …… Date : __/__/__

I declare that I have taken note of the withdrawal of consent by the aforementioned patient and as described above.

Institution: ……

Investigator name (or their representative): ……

Signature:…… Date: __/__/__

The investigator will send the signed form to Julius Clinical within 1 week, attn. Study coordinator GRIPonMASH. Julius Clinical will confirm receipt of the form.

BMJ Open

Global Research Initiative for Patient Screening on MASH protocol (GRIPonMASH): rationale and design of a prospective multicenter study

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	Willemse, José; Liver Patients International Franco, Oscar H; University Medical Centre Utrecht, Julius Center for Health Sciences and Primary Care Grobbee, Diederick; University Medical Centre Utrecht, Julius Center for Health Sciences and Primary Care; Julius Clinical BV, Science Castro Cabezas, Manuel; Franciscus Gasthuis en Vlietland, Department of Internal Medicine; Julius Clinical BV, Science consortium, GRIPonMASH; UMC Utrecht
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Diabetes and endocrinology, Diagnostics, General practice / Family practice, Epidemiology, Nutrition and metabolism
Keywords:	Mass Screening, Hepatology < INTERNAL MEDICINE, Gastroenterology < INTERNAL MEDICINE, INTERNAL MEDICINE, EPIDEMIOLOGY

Global Research Initiative for Patient Screening on MASH protocol (GRIPonMASH): rationale and design of a prospective multicenter study

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ABSTRACT

Introduction

The prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) may be as high as 38% in the adult population with potential serious complications, multiple co-morbidities and a high socioeconomic burden. However, there is a general lack of awareness and knowledge about MASLD and its progressive stages (MASH and fibrosis). Therefore, MASLD is still far underdiagnosed. The "Global Research Initiative for Patient Screening on MASH" (GRIPonMASH) consortium focuses on this unmet public health need. GRIPonMASH will help (primary) health care providers to implement a patient care pathway, as recommended by multiple scientific societies, to identify patients at risk of severe MASLD and to raise awareness. Furthermore, GRIPonMASH will contribute to a better understanding of the pathophysiology of MASLD and improved identification of diagnostic and prognostic markers to detect individuals at risk.

Methods

This is a prospective multicenter observational study in which 10.000 high risk patients (type 2 diabetes mellitus, obesity, metabolic syndrome or hypertension) will be screened in 10 European countries using at least two non-invasive tests (FIB-4 and FibroScan). Blood samples and liver biopsy material will be collected and biobanked, and multi-omics analyses will be conducted.

Ethics and dissemination

The study will be conducted in compliance with this protocol, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), and applicable national and international regulatory requirements. The study initiation package is submitted at the local level. The study protocol has been approved by local medical ethical committees in all 10 participating countries. Results will be made public and published in scientific, peer-reviewed, international journals and at international conferences.

Registration details

Clinicaltrials.gov ID: NCT05651724, registration date: 15 Dec 2022

Strenghts and limitations of this study

1. Multicenter European study embedded in clinical practice
2. Implementation of accepted European guidelines
3. Stimulate collaboration between primary and secondary care
4. Liver biopsies only in subset of patients

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) [1] is a disease with increasing prevalence, associated with metabolic and cardiovascular morbidity [2], [3], [4], [5]. The estimated global prevalence of MASLD is now over 38% [6]. Patients with comorbidities such as type 2 diabetes mellitus (T2DM), obesity, metabolic syndrome and hypertension are at an increased risk for MASLD. Prevalence estimates range up to i.e., 55% in people with T2DM and up to 75% for people with obesity [7], [8], [9]. Overall, the socioeconomic burden and costs of MASLD are high [10], [11]. Despite its prevalence and associated risks, MASLD is often underdiagnosed.

The progressive stages of the MASLD spectrum are metabolic dysfunction-associated steatohepatitis (MASH) and MASH with progressive liver fibrosis, which can eventually lead to liver cirrhosis, liver failure and hepatocellular carcinoma [12]. Approximately 20 to 25% of MASLD patients develop MASH. The estimated prevalence of MASH in the general European adult population is 6-7% [13]. Again, a much higher prevalence has been suggested in i.e., those with T2DM (up to 65%) and obesity (up to 30%) [14]. The severity of MASH is determined by the presence and stage of liver fibrosis, a key determinant of liver-related complications and both liver-related and all-cause mortality [4], [15]. The gold standard for a confirmatory MASH diagnosis and staging is histology by liver biopsy, although non-invasive tests (NITs) are increasingly being used as diagnostic and prognostic tools as well [16], [17].

MASLD is usually asymptomatic and is often not detected until patients enter the progressive stages and/or develop complications, whereas in the early stages the disease is reversible. In the USA, MASH is already the leading cause of liver transplants in women, and the second most common cause in men [18]. Therefore, timely diagnosis and staging of MASLD is important to identify the growing group of patients at increased risk of liver-related and cardiovascular complications. A multidisciplinary approach should be initiated in these patients, which may include intensive lifestyle intervention [19], participation in therapeutic trials and, in selected cases, bariatric surgery [20]. The first drug for this indication (Resmetirom) has been approved by the FDA in March 2024 [21].

Several scientific societies have proposed patient care pathways for implementation in clinical practice to identify patients with severe MASLD [22], [23], [24]. The European Association for the Study of Liver (EASL), the European Association for the Study of Diabetes (EASD) and the European Association for the Study of Obesity (EASO) jointly developed NAFLD clinical practice guidelines in 2016 [16]. In 2021 the first "*Clinical Practice Guidelines on Non-invasive tests for Evaluation of Liver Disease Severity and Prognosis*" were published [23], which proposed a diagnostic flow chart using concordant NITs to screen patients at risk. NITs are generally categorized as blood-based diagnostics (that can be either algorithms based on liver enzymes, or specific serum markers, usually combined) or imaging-based techniques (i.e., ultrasound, magnetic resonance imaging, transient elastography). In June 2024 an updated guideline was published: *"EASL-EASD-EASO Clinical Practice Guidelines on the*

management of metabolic dysfunction-associated steatotic liver disease (MASLD)" reflecting the new nomenclature and recent developments in the field [25]. While the aforementioned and multiple other international guidelines from scientific societies [16], [25], [26], [27], [28], urge to screen individuals at high risk of developing progressive stages of MASLD, these recommendations have not been broadly implemented [29], [30], [31]. There are several reasons for this: MASLD is a silent disease, there is lack of awareness among professionals for MASLD and its associated risks [32], and no pharmacological therapeutic intervention is available in Europe yet.

To assist (primary) health care providers to implement the patient care pathway as suggested by the EASL [23], [25], the "Global Research Initiative for Patient Screening on MASH" (GRIPonMASH) study has been designed. Within this study, high risk patients identified at primary care practices or attending hospital outpatient clinics will be screened for the presence of MASLD, liver fibrosis and (at-risk) MASH using at least 2 NITs in 10 European countries. Additional published and exploratory NITs will also be investigated [33]. Furthermore, a central biobank will be set up and multi-omics will be applied to gain a better understanding of the pathophysiology of MASLD-MASH, and to evaluate diagnostic and prognostic markers to identify patients at risk. Simultaneously, GRIPonMASH aims to promote awareness and implementation of screening among (primary) health care providers, thereby improving clinical care.

METHODS AND ANALYSIS

Study design

In this prospective multicenter, observational study the implementation of a transmural patient care pathway will be promoted. The study will initially employ 2 NITs: 1) the most commonly used blood-based diagnostic, the Fibrosis-4 index (FIB-4); and 2) liver stiffness measurement by vibration-controlled transient elastography (LSM by VCTE) using the FibroScan®. In total 10.000 adult patients at high risk to develop MASLD and MASH (i.e., having T2DM, metabolic syndrome, obesity or arterial hypertension) will be screened. See Figure 1 for an overview of the study design.

Patient care pathway and return to standard care

Participants will be recruited at consultation hours at participating primary care centers and outpatient clinics. Prospective participants will be invited to the Center of Excellence (CoE) for the first visit. Informed consent will be obtained from all individual participants included in the study (Master Patient Information File included as Supplemental Material I). Participants will be instructed to come to each visit after an overnight fast. The assessments of the first visit include baseline clinical characteristics, blood sampling, a FibroScan examination and a lifestyle assessment (by patient-reported outcome (PRO) surveys and an optional 24-hour dietary recall). The participants and referring (primary) health care providers will receive feedback on the FibroScan results and the next steps to take according to the patient care pathway. When the LSM by VCTE results are indeterminate (LSM 8 – 12 kPa) lifestyle recommendations will be provided to the participant.

The patient care pathway provides 3 management options based on the LSM by VCTE results. Option 1) if the FibroScan examination indicates low risk of advanced fibrosis (LSM < 8 kPa), the participant returns to standard care. Option 2) when the FibroScan examination is indeterminate (LSM 8 – 12 kPa), the FibroScan examination and blood sampling will be repeated after 12 weeks (visit 2). Depending on the re-test the participant either returns to standard care (if LSM < 8 kPa) or will be referred to a specialist at the MASLD clinic for detailed analysis (if LSM ≥ 8 kPa). Alternative diagnoses will be excluded as described in the EASL guideline [16], [25], blood will be sampled and a liver biopsy is offered to definitively diagnose presence of MASH and the fibrosis stage, following local clinical care protocols (visit 3). If MASH is not confirmed after detailed analysis, the participant will return to their referring care provider. If MASH is confirmed, the participant will stay at the MASLD clinic for follow-up and possible treatment according to the most recent local guidelines. Patients fulfilling the criteria to undergo bariatric surgery, should receive this recommendation if the procedure is available in the participating country/CoE. If during the study, pharmaceutical therapies become available for this indication, these should be prescribed following current local practice. Option 3) when the FibroScan examination indicates advanced fibrosis (LSM > 12 kPa), the participant will be referred to a specialist at the MASLD clinic for detailed analysis according to local clinical care protocols (visit 3). In these cases a liver biopsy is also strongly advised, unless there is already evidence of liver decompensation. The clinical follow-up of patients at the MASLD clinic is part of standard clinical care, including the local reading of liver biopsies.

Long-term follow-up

All participants will be included in a long-term follow-up program. The European guidelines recommend to retest patients at risk every 1-5 years [23], [25]. At 3 and 5 years after inclusion date, all participants are invited for a follow-up visit which includes a FibroScan examination, fasted blood sampling, lifestyle assessment and a follow-up questionnaire.

Study duration

The expected study duration is 8 years, from June 2023 to March 2031. After the pre-screening at the (primary) health care provider, a participant should attend the first visit at the CoE within 4 weeks. Within 2 weeks, the referring care provider should receive feedback from the CoE with the results of the first visit and recommendation for follow-up according to the patient care pathway. In the case of LSM > 12 kPa at visit 1, the participants should receive an appointment with the specialist at the MASLD clinic within 16 weeks for further detailed analysis and a possible liver biopsy. In the case of LSM ≥ 8 kPa at retest (visit 2), the participants should receive an appointment at with the specialist at the MASLD clinic within 30 weeks for further detailed analysis and a possible liver biopsy. If available, earlier consultation is allowed. Participants should be fully evaluated within a period of 8 months after referral and feedback needs to be sent to the referring care provider.

Study population

This study will be conducted in adult patients (over 18 years of age) with a current or prior diagnosis of at least one of the following four conditions: T2DM, metabolic syndrome, obesity or arterial hypertension. Participants must meet the following inclusion criteria: 1) being willing to provide written informed consent; 2) 18-75 years of age; 3) either diagnosed with or currently being treated for at least one of the earlier mentioned conditions according to the criteria provided in Table 1.

Table 1: Inclusion criteria, based on criteria for diagnosis of type 2 diabetes mellitus, obesity, arterial hypertension and metabolic syndrome. HCP = health care provider.

Condition	Criteria	
Type 2 diabetes mellitus	At least 2 times a fasting glucose > 7,0 mmol/L	
	Or elevated non-fasting glucose >11,1 mmol/L 2 hrs after OGT	
	Or HbA1c ≥48 mmol/mol (≥6.5%)	
	Or being actively treated for previously diagnosed type 2 diabetes by a HCP	
Obesity	Body mass index (BMI) ≥ 30 kg/m²	
	Or waist circumferences: *Caucasian: male ≥ 94 cm, female ≥ 80 cm* *South-Asian/Chinese: male ≥90 cm, female ≥80 cm* *Japanese: male ≥85 cm, female ≥90 cm*	
Arterial hypertension	Systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg	
	Or being actively treated for previously diagnosed arterial hypertension by a HCP	
Metabolic syndrome	Central obesity defined with waist circumference (see above) *if BMI is ≥30 kg/m², central obesity can be assumed and waist circumference does not need to be measured*	
	AND any two of the following:	
	Raised triglycerides	≥ 150 mg/dL (1.7 mmol/L) *or specific treatment for this lipid abnormality*
	Reduced HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in males < 50 mg/dL (1.29 mmol/L) in females *or specific treatment for this lipid abnormality*
	Raised blood pressure (BP)	Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg *or treatment of previously diagnosed hypertension*
	Raised fasting plasma glucose (FPG)	FGP ≥ 100 mg/dL (5.6 mmol/L) 2h OGTT ≥ 7.8 mmol/L or previously diagnosed type 2 diabetes *(if above >5.6 mmol/L or 100 mg/dL, an oral glucose tolerance test is strongly recommended, but is not necessary to define presence of the syndrome)*

A participant who meets any of the following criteria will be excluded from participation in this study: 1) patient with known hepatitis B, C or HIV or any other liver condition (like hemochromatosis, sarcoidosis, Wilson's disease, etc); 2) patient with any other condition that may lead to liver fibrosis or cirrhosis; 3) patient engages in (excessive) alcohol use (defined as: > 3 units/day [30 grams/day] in males and > 2 units/day [20 grams/day] in females; 4) patient with history or evidence of any other clinically significant condition or planned or expected procedure that in the opinion of the investigator, may compromise the patient's safety or ability to be included in this study; 5) the patient is an

employee or contractor of the facility that is conducting the study or is a family member of the investigator, sub-Investigator, or any sponsor personnel; 6) the patient is not able to understand the details of the protocol and/or is not able to provide written informed consent; 7) the patient is pregnant or breastfeeding; 8) the patient underwent bariatric surgery in the last 12 months.

Study sites

The 10 selected countries are: Belgium, Czech Republic, France, Germany, Greece, Italy, Portugal, Romania, Spain and The Netherlands. In each country clinics defined as CoE and several satellite primary care centers will be recruited. The general aim is to recruit 1-2 CoE per country depending on the local size and distribution of primary care centers and CoEs. CoEs should fulfil the following criteria: 1) CoE should be able to perform liver biopsies; 2) CoE should have personnel trained to carry out FibroScan examinations. XL probe needs to be available to be used when indicated by FibroScan; 3) CoE should be able to store blood and liver samples for a limited time; 4) CoE should be able to arrange shipment of samples by courier services to the central biobank; 5) CoE should have sufficient access to (at least 2) primary care centers willing to participate in GRIPonMASH including at least 100 patients per practice; 6) the team of CoE should at least comprise: 1 internist/diabetologist or endocrinologist; 1 gastroenterologist/hepatologist.

Biobank

The collected liver and blood samples will be shipped to a central biobank at UMC Utrecht, the Netherlands. Participants will be asked to approve of biobanking of their blood, DNA and liver samples for a total of 15 years, since we foresee rapid developments in available diagnostic tools and multi-omics methods. Some samples will be used for pre-specified central assessments. We aim to analyse the remaining samples over the next decade using any novel scientific advances.

Sample size estimation

The primary objectives of this study are to establish the prevalence of MASLD, at-risk MASH and liver fibrosis based on FibroScan examinations in high-risk patients, to compare the prevalence between countries and assess the added value of a 2-step pathway (see Table 4). As such, this study should be considered a pilot study helping to implement clinical guidelines and consolidate patient care pathways. We estimate that including 1000 patients per country will give a realistic reflection of the situation per country and that it will provide enough power to determine differences between included countries and diagnostics (see Figure 2).

Study procedures

Randomisation, blinding and treatment allocation will not occur in this study, as participants will follow the flow of the patient care pathway. Treatment of T2DM, metabolic syndrome, obesity and/or arterial hypertension will continue according to routine care.

Baseline characteristics

At the first visit the following data will be recorded: age, gender, ethnicity, primary diagnosis for inclusion and date of diagnosis, comorbidities, short medical history, short family history and current medication use. A short physical exam will be performed including: height, weight, waist circumference and blood pressure. If the participant does not recall medication use, the investigator will request this information from the care provider. Results of recent (not older than 6 months) laboratory measurements of urine albumin/creatinine ratio and presence of microalbuminuria will be requested from the care provider, if available.

FibroScan examination

All patients will undergo a FibroScan (Echosens, France) examination to assess simultaneously LSM by VCTE and controlled attenuation parameter (CAP™). The measurements will be carried out in participants fasting for at least three hours and after a 5-minute resting time in the supine position on the examination bed by a trained and certified staff member of the CoE. Tables 2 and 3 provide the cut-off values that will be used to interpret LSM and CAP respectively. CAP can be used to estimate steatosis and LSM to estimate fibrosis. Type of probe used and validity of measurements will also be recorded.

Table 2: cut-off values for controlled attenuation parameter (CAP) [25], [34], [35].

CAP (dB/m)	Indication of steatosis stage
248 – 267	S1
268 – 279	S2
≥ 280	S3

Table 3: cut-off values for liver stiffness measurement (LSM) by vibration-controlled transient elastography [25], [35], [36].

LSM by VCTE (kPa)	Indication fibrosis stage
< 8	Low risk of advanced fibrosis
8 – 12	Indeterminate risk
> 12	Advanced fibrosis

The recommendations for the next steps in the patient care pathways are based on the LSM by VCTE results, which provides an indication of the fibrosis stage of the participant. The referring care provider will be informed about the results and recommendations following a predefined format.

Blood sampling and processing

All blood samples will be collected after an overnight fast. At visit 1, 47,5 mL of blood will be drawn an processed to allow biobanking, direct central analysis and local measurements. 6 mL blood, 4 plasma aliquots and 7 serum aliquots will be biobanked. 2 serum aliquots and 2 mL blood in a PAXgene tube will be shipped directly to central analysis. The CoE will perform local measurements of aspartate aminotransferase (AST) level, alanine transaminase (ALT) level, platelet count and Hba1c. At visit 2, 8,5 mL blood will be drawn an processed into 1 plasma aliquot and 2 serum aliquots for biobanking and 1 PAXgene tube for central analysis. At visit 3 and the follow-up visits, 16,5 mL blood will be drawn and processed into 2 plasma aliquots and 3 serum aliquots for biobanking and 1 PAXgene tube

for central analysis. Each CoE will receive 'biobank kits' including tubes and pre-printed labels to ensure high-quality sample collection that is comparable across sites. The samples processed for biobanking will be stored locally at -80 degrees Celsius and then be shipped from the CoE to the central biobank in batches.

Fibrosis-4 index (FIB-4)

The FIB-4 is a widely used blood-based diagnostic [23], [37], [38]. The FIB-4 index is an algorithm based on age, AST, ALT and platelet count and is calculated at baseline using the following formula: FIB-4 = (AGE (years) * AST (U/L)) / (platelet count (10^9/L) * square root(ALT (U/L))).

FibroScan-AST (FAST) score

The FibroScan-AST (FAST) score is an algorithm combining the FibroScan examination results (LSM by VCTE and CAP) with a blood marker (AST) to identify patients with active fibrotic-MASH, also referred to as at-risk MASH [39].

Liver biopsy

Liver biopsies will be carried out according to local procedures at the participating CoE. The CoE will prepare: 1) slides for local reading according to local standard operating procedures; 2) three unstained slides to allow central reading and digital pathology assessment; and 3) snap freeze liver tissue with liquid nitrogen to allow metabolomic analysis. These tissue samples will be temporarily stored at the CoE before shipment to the central biobank. A panel of 2 independent and well-trained pathologists will execute the central reading. The histopathological features of MASH (steatosis, hepatocyte ballooning, lobular inflammation) will be assessed using the NAS criteria [40] and Steatosis, Activity, and Fibrosis (SAF) score [41], and the fibrosis stage will be determined.

Baseline laboratory measurements (central)

The following variables will be measured centrally for each participant. Metabolic panel: total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), glucose, creatinine. Liver enzymes: alanine transaminase (ALT), aspartate aminotransferase (AST), bilirubin (direct and total), gamma-glutamyltransferase, alkaline phosphatase. Other: lipoprotein (a) (Lp(a)), insulin, sex hormone-binding globulin (SHBG), free thyroxine (FT4), tri-iodothyronine (T3), thyroid stimulating hormone (TSH), albumin and globulins.

Genetic and proteomic analysis (central)

DNA will be extracted from the biobanked full blood samples. Genetic analyses of patients will be accomplished by genome-wide association studies (GWAS). Targeted and untargeted proteomics analysis will be used to identify serological protein biomarkers.

Metabolomic, lipidomic and fluxomic analysis (central)

Metabolomics, lipidomics and fluxomics studies will be performed on the liver biopsy and fasted blood samples. Metabolites including lipids will be extracted from EDTA plasma samples and analysed using mass-spectrometry (MS) based metabolomics and lipidomics methods. A global metabolomics high-resolution platform will be used allowing the targeted and untargeted/global analyses of a wide range of hundreds of small molecules including amino acids, central energy and carbon metabolism, but also exogenous molecules dietary chemicals, microbiome-derived metabolites, environmental chemicals, commercial products, and drugs. A lipidomics platform allows the analysis of more than 20 lipid classes including diglycerides, triglycerides, phospholipids, ceramides, sphingolipids and many more. A targeted MS/MS platform will be used covering more than 300 modified free fatty acids acting as bioactive lipid mediators both locally and systemically which are involved in immunology (innate immunity), inflammation, chemotaxis, cell survival, cell proliferation and differentiation. Fluxomic measurement based on metabolites labelled e.g., with 13C-glucose or 2H2O will be performed in vitro and in vivo to quantify metabolic fluxes and elucidate pathophysiological mechanisms.

Biomarker analysis (central)

For the identification of novel circulating biomarkers, a panel of (potential) biomarkers will be identified in vitro by using liver-on-a-chip tools. Additionally, potential novel biomarkers (i.e., PLIN-2 [HeparDx], PRO-C3, PRO-C6, oxidized low-density lipoprotein [LDL], glucagon, gastric inhibitory polypeptide [GIP], glucagon-like peptide-1 [GLP-1], FICE34, TLM3) will be measured.

Lifestyle phenotyping

Lifestyle will be phenotyped in 7 different areas: diet, water consumption, alcohol consumption, physical activity, smoking, sleep and endocrine environmental disruptors. The following self-reported questionnaires are used: diet will be assessed using the Mediterranean Diet Score (PREDIMED) [42]; water consumption will be obtained with one question; alcohol consumption, apart from wine in the PREDIMED, will be obtained with a maximum of 3 semi-closed questions; physical activity will be recorded using the International Physical Activity Questionnaire – Short Form (IPAQ-SF) [43]; smoking habits will be obtained with a maximum of 8 multiple and semi-closed question; sleep quality will be obtained with Brief version Pittsburgh Sleep Quality Index (B-PSQI) [44]; endocrine disrupting chemical exposures will be evaluated using 6 questions. Additionally, in CoEs with a dietician and resources available, a 24-hour dietary recall will be annotated by an interviewer on 2 days (most recent regular weekday and one weekend day).

Lifestyle recommendations

Lifestyle recommendations will be provided based on current clinical guidelines for MASLD/MASH patients [25], [45]. A member of the study team will recommend participants adhere to a healthy dietary pattern and level of physical activity. The aim of the lifestyle recommendations is to lose 5% weight (ideally 10%); however, losing weight should not be the participant's ultimate goal but rather their adherence to a healthy lifestyle.

Long-term follow-up (at 3 and 5 years)

A follow-up questionnaire has been designed to inquire about the disease status and comorbidities, possible liver-related and non-liver related complications and changes in medication of the participant. At the follow-up visits at 3 and 5 years the FibroScan examination will be repeated. At the same visit, the follow-up questionnaire should be completed by the research team who will request the necessary information from the participant's care provider. At both timepoints the participants will be asked to complete the lifestyle assessment again.

Table 4: primary and secondary objectives, subobjectives and associated outcome measures.

PRIMARY OBJECTIVE		
Objective	**Sub-objectives**	**Outcome measures**
To implement a transmural patient care pathway, to identify patients with MASLD and its progressive form of MASH in primary care centers and clinics in 10 European countries	Prevalence of liver steatosis and MASLD estimated by FibroScan CAP in patients at risk	- Steatosis stage deduced from CAP measurement with FibroScan - MASLD definition (CAP > 280 and 1 out of 5 cardiometabolic risk factors)
	Prevalence of liver fibrosis estimated by FibroScan LSM in patients at risk	Fibrosis stage deduced from LSM by VCTE measurement with FibroScan
	Prevalence of at-risk MASH estimated by FAST score in patients at risk	At-risk MASH deduced from FAST score
	Prevalence of MASH in patients at risk (in subset only)	MASH diagnosis confirmed by histology (NAS/SAF criteria) upon liver biopsy
	Comparison of the prevalence of liver steatosis, MASLD, liver fibrosis and (at-risk) MASH between the participating countries	Prevalence (see above) stratified per country
	Added value of a 2-step pathway (FIB-4 + FibroScan) as compared to FibroScan only for detection of high-risk patients	Number of patients at risk identified by FIB-4 in comparison to numbers found using LSM by VCTE with FibroScan, and numbers found in combination
SECONDARY OBJECTIVES		
Objective	**Sub-objectives (if applicable)**	**Outcome measures**
To gain a better understanding of the pathophysiology of MASLD and to identify markers that will help to detect patients at risk (by applying a multi-omics approach)	Build/validate a diagnostic model to identify MASH patients in a high-risk population	Possible model parameters are all baseline clinical characteristics reported in the eCRF
	Explore genotypes related to MASH in different European countries	Genomic (GWAS) and proteomics analysis on collected blood samples
	Explore (non-invasive) metabolite biomarkers identifying MASH in patients at risk	Mass-spectrometry (MS) based metabolomic and lipidomic analyses and fluxomics analysis on collected blood samples, both targeted and untargeted approaches
	Prevalence of co-morbidities and associated therapies (especially for cardiovascular disease) in patients with MASH compared to those without, in high risk patient populations.	Prevalence of comorbidities in patients at- risk, patients diagnosed with MASLD/MASH
	Identify prognostic factors/biomarkers for complications in patients with MASLD and MASH at 5 years	Disease progression and liver-related and non-liver related complications
Evaluate Patient Reported Outcomes (PRO) from baseline throughout follow-up		PRO surveys at baseline and follow-up

Confirm high risk of liver fibrosis based on second FibroScan examination after lifestyle recommendations (in subset only)	Revisited FibroScan examination (Cap and LSM by VCTE) 12 weeks after first FibroScan and after lifestyle recommendations
To evaluate changes in CAP, LSM by VCTE and FAST scores over time	Repeated CAP, LSM by VCTE and FAST measurement over time

Data management

Clinical data will be captured using an electronic case report form (eCRF), only authorised staff at the CoE will be allowed to enter data into the eCRF. All eCRF data should be verifiable to a source at the CoE or accessible by the site staff, except if direct entry was allowed. Participants are identified by a code, only the local investigators will have the key. The investigator(s) will be responsible for ensuring eCRF data completeness and accuracy. Source data verification and data quality will be assessed by remote eCRF reviews, statistical checks and in-person monitor visits. Data from the eCRF and other sources (i.e., central analyses, Liver Health Management platform) will be encoded and stored in a central study database (Data Science Platform).

Statistical analysis

R will be used for statistical analysis, p-values < 0.05 (2-tailed) are considered statistically significant. By default, parametric testing will be employed. In case of not normally distributed variables, non-parametric tests will be employed. Before analysis, we will assess the degree and possible reasons for missingness and apply appropriate methods to handle missing data i.e., multiple imputation.

As the primary objective is the implementation of a patient care pathway (see table 4), primarily descriptive statistics will be used instead of hypothesis testing. Prevalence of MASLD, liver steatosis, fibrosis and (at-risk) MASH will be reported for each country. The prevalence will be compared between countries using one-way ANOVA (or Kruskal Wallis test). The overlap in numbers of patients at risk identified by the 2 NITs will be reported as well.

For the secondary objectives the following statistical methods will be used: diagnostic modelling using logistic regression and machine learning (ML)/artificial intelligence (AI) approaches; associations in GWAS and proteomics data will be analysed using logistic regression; associations between MASH diagnosis by histology and (non-invasive) metabolite (bio)markers will be analysed using logistic regression; prevalence of co-morbidities will be estimated in total group of patients at risk with/without MASLD, the difference between the groups will be analysed using one-way ANOVA (or Kruskal Wallis test); prognostic modelling using logistic regression and ML/AI approaches; mixed linear modelling will be implemented to assess changes in PRO over time; difference between 1st and 2nd LSM by VCTE measurements will be assessed using a paired t-test (or Wilcoxon signed rank test); mixed linear modelling will be implemented to assess the repeated measurements of CAP, LSM by VCTE and FAST over time.

Ethics and dissemination

The study will be conducted in compliance with this protocol, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), and applicable national and international regulatory requirements (including Declaration of Helsinki). The study initiation package is submitted at the local level. The study protocol has already been approved by local medical ethical committees in all 10 participating countries: Netherlands (Medical Research Ethics Committees United , A23.273/R22.057), Germany (Etik-Kommission Landesartzekammer Rheinland-Pfalz, 2022-16602 and Ärtzekammer des Saarlandes, 185/24), Spain (Comités Éticos de Investigación de los Hospitales Universitarios Virgen Macarena y Virgen del Rocío, 1631-N-22), Belgium (Ethische Commissie Universitair Ziekenhuis Antwerpen, EDGE 002626 and Comité d'Ethique C Hôpital Erasme-ULB, SRB2023083), Portugal (Comissao de Etica do Centro Académico de Medicina de Lisboa, 203/23), Greece (Research Ethics & Deontology Committee of HUA, r-3549/08.10.2024) , Romania (Comisia de Etica a Spitalului Municipal Sacele, 7201/13.11.2024), Czech Republic (Etická komise Všeobecné fakultní nemocnice v Praze, 152/24 S Grant) and Italy (Comitato Etico Territoriale Lazio AREA 3, ID 7135) and France (Comité de protection des personnes Ouest IV, 2025-A00083-46). Results will be made public and published in scientific, peer-reviewed, international journals and at international conferences.

Patient and Public Involvement

The draft protocol was reviewed and endorsed by Liver Patients International (LPI). The European Liver Patients Association (ELPA), the European Atherosclerosis Society (EAS) and the European Association for the Study of the Liver (EASL) have also endorsed the final project.

DISCUSSION

Although several factors are involved in the progression from MASLD to MASH (environmental, dietary, genetic), the exact mechanisms behind MASLD development and progression remain unknown [46]. The widely used estimate of 38% worldwide prevalence of MASLD is based on a meta-analysis of studies from 2016 to 2019 [6]. However, exact data about the prevalence in different European countries using accepted diagnostic tools like LSM by VCTE or the golden standard, liver biopsy, are still lacking. This study will provide prevalence estimates based on a large number of participants.

The patient care pathway used in this study is based on the 2021 and 2024 updates of the EASL clinical practice guidelines [23], [25]. In contrast to these guidelines, in the current protocol the two NITs (FIB-4 and FibroScan) will be performed in the same visit. Recently there has been debate about the accuracy of FIB-4 to (pre-)screen in the general population at primary care level [47], as the index was validated and developed in secondary and tertiary care. This issue was acknowledged by the EASL in 2021 and they already advised not to base diagnoses on one single NIT, but to use concordant NITs instead [23]. Therefore, in the current protocol it was decided to perform the

FibroScan and blood sampling for FIB-4 at the same day for each patient. We can then compare the added value of FIB-4 versus FibroScan and their prognostic capacity at follow up within a population at risk identified at the primary care level and outpatient clinics, and thereby either validate the guidelines or propose adjustments.

In line with the EASL Clinical Practice Guidelines, structured programmes aimed at lifestyle changes towards healthy diet and habitual physical activity are advisable [16]. The current study only includes lifestyle recommendations to a subset of participants as this would be feasible within current clinical practice. The level of adherence to the Mediterranean diet will be evaluated throughout the study. The Mediterranean diet is thought to be beneficial [48], although evidence is still limited [49]. We also aim to build towards personalized lifestyle advice.

European experts have demonstrated that most countries in Europe are not yet ready to face the growing MASLD/MASH problem, because they lack guidelines, registries and multidisciplinary programs [30]. Therefore, initiatives such as this study aimed at increasing knowledge and awareness of MASLD and MASH among professionals are essential. In addition, this study aims to integrate all levels of clinical care, which is much needed for multidisciplinary management of MASLD.

Author contributions

DEG and MCC conceptualized the study. VDJ and MCC were the main authors of the manuscript and protocol. DEG, OHF and the members of the initial Julius Clinical-initiated Scientific Steering Committee (HCP, SF, CM, MRG, MET, AGH, LS, JMS, JWMM, AG, VR) and Independent Advisory Board (JW, MA) critically reviewed the initial protocol. TH contributed to the lipidomics/metabolomics section, JV and MD to the liver biopsy section. CFP, RB, GVD, LM, VR, DCS critically review the revised protocol. MCC is guarantor. This study is executed within the GRIPonMASH consortium; the consortium reviewed the protocol.

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Competing interests statement

JMS: Consultant: Akero, Alentis Therapeutics, Astra Zeneca, 89Bio, Boehringer Ingelheim, GSK, Ipsen, Inventiva Pharma, Madrigal, MSD, Northsea Therapeutics, Novartis, Novo Nordisk, Pfizer, Roche, Sanofi, Siemens Healthineers. Research Funding: Boehringer Ingelheim, Siemens Healthcare

GmbH. Stock Options: AGED diagnostics, Hepta Bio. Speaker Honorarium: Gilead Sciences, Advanz, Echosens, MedPublico GmbH.

CM: Consulting fees: Julius Clinical, Echosens, Gilead.

AG: consultancy to Boehringer Ingelheim, Eli Lilly and Company, Metadeq Diagnostics; has participated in advisory boards for: Boehringer Ingelheim, Merck Sharp & Dohme, Novo Nordisk, Metadeq Diagnostics and Pfizer; speaker honorarium or other fees from: Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk and Pfizer.

The other authors have no relevant financial or non-financial interests to disclose.

Provenance and peer review

The protocol was peer reviewed by the members of the initial Julius Clinical-initiated GRIPonMASH Scientific Steering Committee and Principal Investigators of the CoEs. The authors are thankful to Prof. J. Ordovas for providing critical feedback on the final version of this manuscript.

Data availability statement

Collected data will be available upon reasonable request. Only unidentified data are available to researchers who submit a methodologically sound proposal. Proposals may be submitted to the GRIPonMASH consortium for review. The consortium reserves the right to analyse data for development and intellectual property purposes prior to public disclosure.

REFERENCES

[1] M. E. Rinella *et al.*, 'A multi-society Delphi consensus statement on new fatty liver disease nomenclature.', *Hepatology*, Jun. 2023, doi: 10.1097/HEP.0000000000000520.

[2] C. D. Byrne and G. Targher, 'NAFLD: a multisystem disease', *J Hepatol*, vol. 62, no. 1 Suppl, pp. S47–S64, 2015, doi: 10.1016/J.JHEP.2014.12.012.

[3] G. Targher, C. D. Byrne, A. Lonardo, G. Zoppini, and C. Barbui, 'Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis', *J Hepatol*, vol. 65, no. 3, pp. 589–600, Sep. 2016, doi: 10.1016/J.JHEP.2016.05.013.

[4] M. Ekstedt *et al.*, 'Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up', *Hepatology*, vol. 61, no. 5, pp. 1547–1554, May 2015, doi: 10.1002/HEP.27368.

[5] S. Driessen *et al.*, 'Metabolic dysfunction associated steatotic liver disease and the heart', *Hepatology*, Dec. 2023, doi: 10.1097/HEP.0000000000000735.

[6] Z. M. Younossi, P. Golabi, J. M. Paik, A. Henry, C. Van Dongen, and L. Henry, 'The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review.', *Hepatology*, Jan. 2023, doi: 10.1097/HEP.0000000000000004.

[7] J. Quek *et al.*, 'Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis', *Lancet Gastroenterol Hepatol*, vol. 8, no. 1, pp. 20–30, Jan. 2023, doi: 10.1016/S2468-1253(22)00317-X.

[8] L. Henry, J. Paik, and Z. M. Younossi, 'Review article: the epidemiologic burden of non-alcoholic fatty liver disease across the world', *Aliment Pharmacol Ther*, vol. 56, no. 6, pp. 942–956, Sep. 2022, doi: 10.1111/APT.17158.

[9] Z. M. Younossi *et al.*, 'The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis', *J Hepatol*, vol. 71, no. 4, pp. 793–801, Oct. 2019, doi: 10.1016/J.JHEP.2019.06.021.

[10] J. M. Schattenberg *et al.*, 'Disease burden and economic impact of diagnosed non-alcoholic steatohepatitis in five European countries in 2018: A cost-of-illness analysis', *Liver Int*, vol. 41, no. 6, pp. 1227–1242, Jun. 2021, doi: 10.1111/LIV.14825.

[11] T. H. Karlsen *et al.*, 'The EASL–Lancet Liver Commission: protecting the next generation of Europeans against liver disease complications and premature mortality', *The Lancet*, vol. 399, no. 10319, pp. 61–116, Jan. 2022, doi: 10.1016/S0140-6736(21)01701-3/ATTACHMENT/82513520-DAB3-430E-A196-83F57E6FC6C3/MMC1.PDF.

[12] E. E. Powell, V. W. S. Wong, and M. Rinella, 'Non-alcoholic fatty liver disease', Jun. 05, 2021, *Elsevier B.V.* doi: 10.1016/S0140-6736(20)32511-3.

[13] Z. M. Younossi, A. B. Koenig, D. Abdelatif, Y. Fazel, L. Henry, and M. Wymer, 'Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes', *Hepatology*, vol. 64, no. 1, pp. 73–84, Jul. 2016, doi: 10.1002/HEP.28431/SUPPINFO.

[14] J. F. Dufour *et al.*, 'The global epidemiology of nonalcoholic steatohepatitis (NASH) and associated risk factors–A targeted literature review', *Endocrine and Metabolic Science*, vol. 3, p. 100089, Jun. 2021, doi: 10.1016/J.ENDMTS.2021.100089.

[15] A. J. Sanyal *et al.*, 'Prospective Study of Outcomes in Adults with Nonalcoholic Fatty Liver Disease', *N Engl J Med*, vol. 385, no. 17, pp. 1559–1569, Oct. 2021, doi: 10.1056/NEJMOA2029349.

[16] G. Marchesini *et al.*, 'EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease', *J Hepatol*, vol. 64, no. 6, pp. 1388–1402, Jun. 2016, doi: 10.1016/J.JHEP.2015.11.004.

[17] F. E. Mózes *et al.*, 'Performance of non-invasive tests and histology for the prediction of clinical outcomes in patients with non-alcoholic fatty liver disease: an individual participant data meta-analysis', *Lancet Gastroenterol Hepatol*, vol. 8, no. 8, pp. 704–713, Aug. 2023, doi: 10.1016/S2468-1253(23)00141-3.

[18] M. Noureddin *et al.*, 'NASH Leading Cause of Liver Transplant in Women: Updated Analysis of Indications For Liver Transplant and Ethnic and Gender Variances', *Am J Gastroenterol*, vol. 113, no. 11, pp. 1649–1659, Nov. 2018, doi: 10.1038/S41395-018-0088-6.

[19] V. Ratziu, 'Non-pharmacological interventions in non-alcoholic fatty liver disease patients', *Liver International*, vol. 37, pp. 90–96, Jan. 2017, doi: 10.1111/LIV.13311.

[20] M. Ren, X. Zhou, L. Lv, and F. Ji, 'Endoscopic Bariatric and Metabolic Therapies for Liver Disease: Mechanisms, Benefits, and Associated Risks', *J Clin Transl Hepatol*, vol. 10, no. 5, p. 986, Oct. 2022, doi: 10.14218/JCTH.2021.00448.

[21] S. A. Harrison *et al.*, 'A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis', *N Engl J Med*, vol. 390, no. 6, pp. 497–509, Feb. 2024, doi: 10.1056/NEJMOA2309000.

[22] F. Kanwal *et al.*, 'Clinical Care Pathway for the Risk Stratification and Management of Patients With Nonalcoholic Fatty Liver Disease', *Gastroenterology*, vol. 161, no. 5, pp. 1657–1669, Nov. 2021, doi: 10.1053/J.GASTRO.2021.07.049.

[23] A. Berzigotti *et al.*, 'EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update', *J Hepatol*, vol. 75, no. 3, pp. 659–689, Sep. 2021, doi: 10.1016/J.JHEP.2021.05.025.

[24] A. Srivastava *et al.*, 'Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease', *J Hepatol*, vol. 71, no. 2, pp. 371–378, Aug. 2019, doi: 10.1016/J.JHEP.2019.03.033.

[25] E. Association for the Study of the Liver *et al.*, 'EASL–EASD–EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD)', *J Hepatol*, vol. 0, no. 0, Jun. 2024, doi: 10.1016/J.JHEP.2024.04.031.

[26] M. E. Rinella *et al.*, 'AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease.', *Hepatology*, Feb. 2023, doi: 10.1097/HEP.0000000000000323.

[27] K. Cusi *et al.*, 'American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD)', *Endocrine Practice*, vol. 28, no. 5, pp. 528–562, May 2022, doi: 10.1016/J.EPRAC.2022.03.010.

[28] P. Ferenci *et al.*, 'World Gastroenterology Organisation Global Guidelines Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis', 2014. [Online]. Available: www.jcge.com

[29] Q. M. Anstee *et al.*, 'Real-world management of non-alcoholic steatohepatitis differs from clinical practice guideline recommendations and across regions', *JHEP Reports*, vol. 4, no. 1, Jan. 2022, doi: 10.1016/j.jhepr.2021.100411.

[30] J. v. Lazarus *et al.*, 'European "NAFLD Preparedness Index" — Is Europe ready to meet the challenge of fatty liver disease?', *JHEP Reports*, vol. 3, no. 2, p. 100234, Apr. 2021, doi: 10.1016/J.JHEPR.2021.100234.

[31] J. M. Schattenberg, Q. M. Anstee, C. Caussy, E. Bugianesi, and B. Popovic, 'Differences between current clinical guidelines for screening, diagnosis and management of nonalcoholic fatty liver disease and real-world practice: a targeted literature review', 2021, *Taylor and Francis Ltd.* doi: 10.1080/17474124.2021.1974295.

[32] S. Driessen *et al.*, 'A global survey of health care workers' awareness of non-alcoholic fatty liver disease: The AwareNASH survey', *United European Gastroenterol J*, 2023, doi: 10.1002/UEG2.12445.

[33] Y. Vali *et al.*, 'Biomarkers for staging fibrosis and non-alcoholic steatohepatitis in non-alcoholic fatty liver disease (the LITMUS project): a comparative diagnostic accuracy study', *Lancet Gastroenterol Hepatol*, vol. 8, no. 8, pp. 714–725, Aug. 2023, doi: 10.1016/S2468-1253(23)00017-1.

[34] P. J. Eddowes *et al.*, 'Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease', *Gastroenterology*, vol. 156, no. 6, pp. 1717–1730, May 2019, doi: 10.1053/J.GASTRO.2019.01.042.

[35] V. W. S. Wong *et al.*, 'Unified interpretation of liver stiffness measurement by M and XL probes in non-alcoholic fatty liver disease', *Gut*, vol. 68, no. 11, pp. 2057–2064, 2019, doi: 10.1136/GUTJNL-2018-317334.

[36] W. Theel *et al.*, 'Evaluation of nonalcoholic fatty liver disease (NAFLD) in severe obesity using noninvasive tests and imaging techniques', *Obesity Reviews*, vol. 23, no. 8, p. e13481, Aug. 2022, doi: 10.1111/OBR.13481.

[37] J. v Lazarus *et al.*, 'Real-world evidence on non-invasive tests and associated cut-offs used to assess fibrosis in routine clinical practice', *JHEP Reports*, vol. 0, no. 0, p. 100596, Sep. 2022, doi: 10.1016/J.JHEPR.2022.100596.

[38] E. J. Grobbee *et al.*, 'Improvement of non-invasive tests of liver steatosis and fibrosis as indicators for non-alcoholic fatty liver disease in type 2 diabetes mellitus patients with elevated cardiovascular risk profile using the PPAR-α/γ agonist aleglitazar', *PLoS One*, vol. 17, no. 11, p. e0277706, Nov. 2022, doi: 10.1371/JOURNAL.PONE.0277706.

[39] P. N. Newsome *et al.*, 'FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study', *Lancet Gastroenterol Hepatol*, vol. 5, no. 4, pp. 362–373, Apr. 2020, doi: 10.1016/S2468-1253(19)30383-8.

[40] A. C. Sheka, O. Adeyi, J. Thompson, B. Hameed, P. A. Crawford, and S. Ikramuddin, 'Nonalcoholic Steatohepatitis: A Review', *JAMA*, vol. 323, no. 12, pp. 1175–1183, Mar. 2020, doi: 10.1001/JAMA.2020.2298.

[41] F. Nascimbeni *et al.*, 'Clinical validation of the FLIP algorithm and the SAF score in patients with non-alcoholic fatty liver disease', *J Hepatol*, vol. 72, no. 5, pp. 828–838, May 2020, doi: 10.1016/J.JHEP.2019.12.008.

[42] M. A. Martínez-González *et al.*, 'A 14-Item Mediterranean Diet Assessment Tool and Obesity Indexes among High-Risk Subjects: The PREDIMED Trial', *PLoS One*, vol. 7, no. 8, Aug. 2012, doi: 10.1371/JOURNAL.PONE.0043134.

[43] C. L. Craig *et al.*, 'International physical activity questionnaire: 12-country reliability and validity', *Med Sci Sports Exerc*, vol. 35, no. 8, pp. 1381–1395, Aug. 2003, doi: 10.1249/01.MSS.0000078924.61453.FB.

[44] C. Sancho-Domingo, J. L. Carballo, A. Coloma-Carmona, and D. J. Buysse, 'Brief version of the Pittsburgh Sleep Quality Index (B-PSQI) and measurement invariance across gender and age in a population-based sample', *Psychol Assess*, vol. 33, no. 2, pp. 111–121, Feb. 2021, doi: 10.1037/PAS0000959.

[45] S. M. Francque *et al.*, 'Non-alcoholic fatty liver disease: A patient guideline'.

[46] Q. M. Anstee *et al.*, 'Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort☆', *J Hepatol*, vol. 73, no. 3, pp. 505–515, Sep. 2020, doi: 10.1016/J.JHEP.2020.04.003.

[47] I. Graupera *et al.*, 'Low Accuracy of FIB-4 and NAFLD Fibrosis Scores for Screening for Liver Fibrosis in the Population', *Clin Gastroenterol Hepatol*, vol. 20, no. 11, pp. 2567-2576.e6, Nov. 2022, doi: 10.1016/J.CGH.2021.12.034.

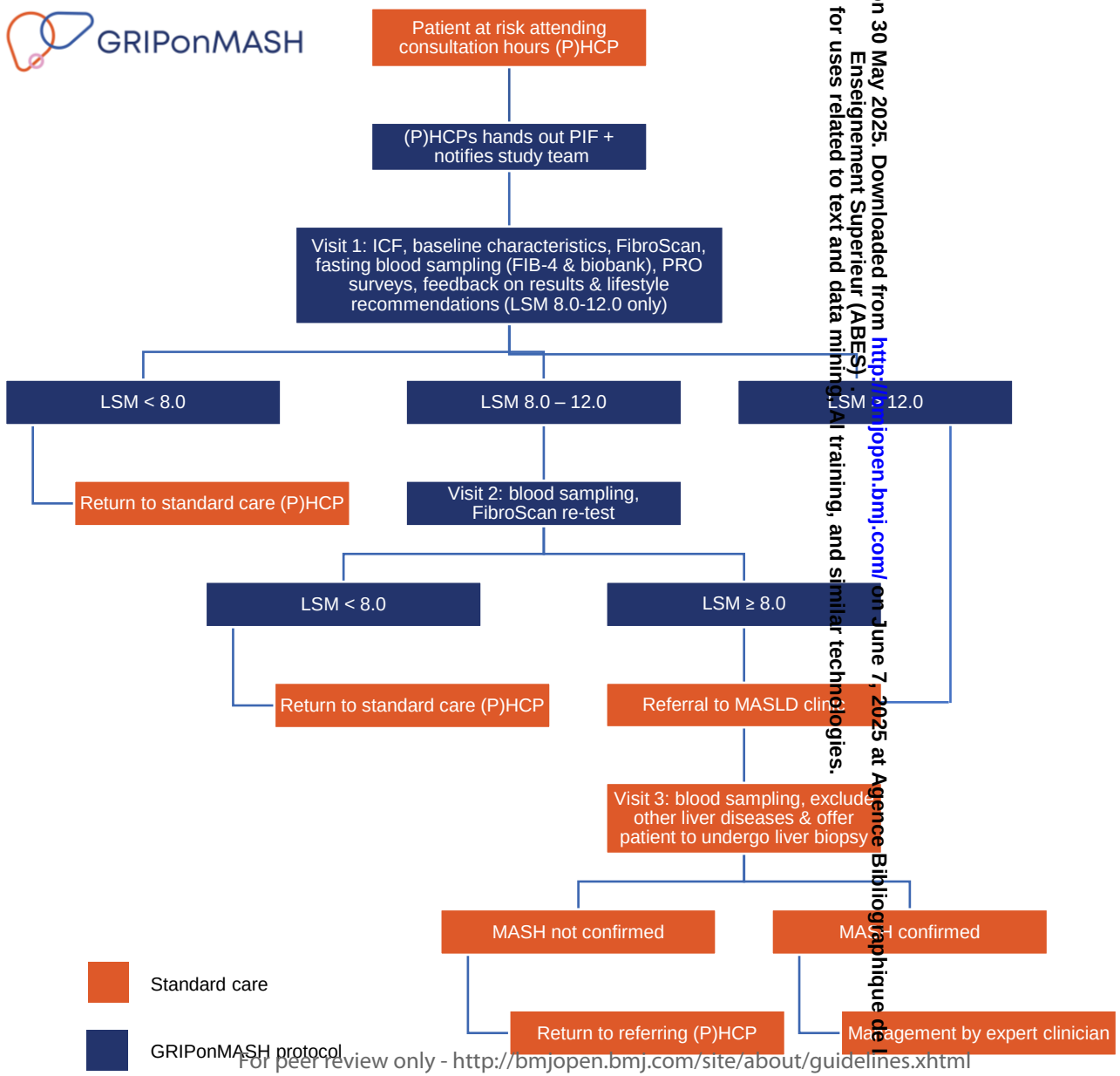
[48] Y. Gepner *et al.*, 'The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content', *J Hepatol*, vol. 71, no. 2, pp. 379–388, Aug. 2019, doi: 10.1016/J.JHEP.2019.04.013.

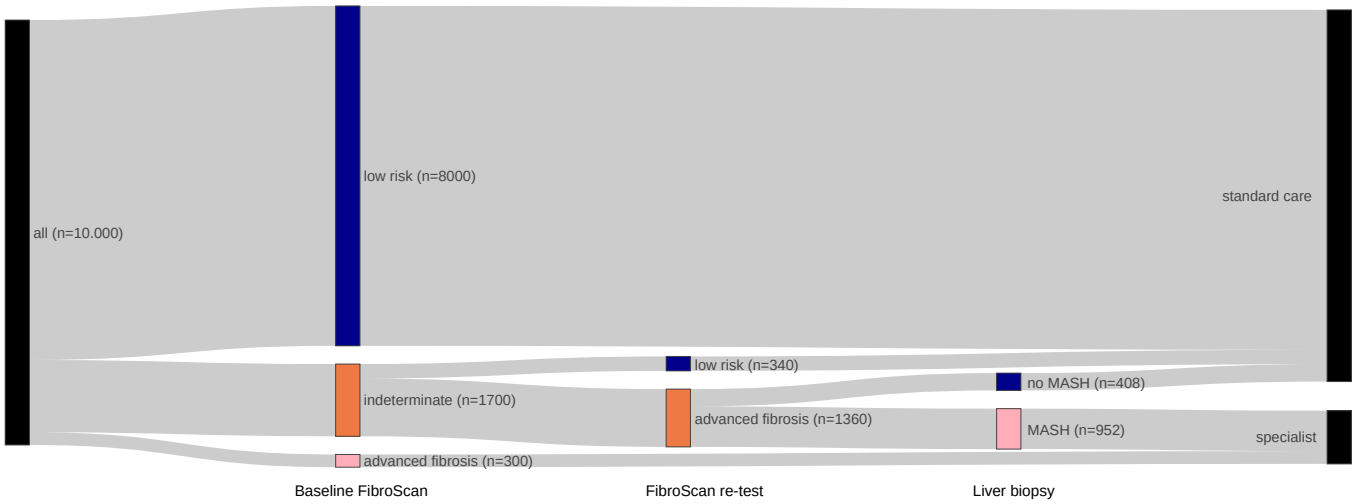
[49] M. C. P. Torres *et al.*, 'Mediterranean Diet and NAFLD: What We Know and Questions That Still Need to Be Answered.', *Nutrients*, vol. 11, no. 12, pp. E2971–E2971, Dec. 2019, doi: 10.3390/NU11122971.

Figure legends

Figure 1: Overview of study design and patient flow. Abbreviations used: (P)HCP = (primary) health care provider, PIF = patient information folder, ICF = informed consent form, FIB-4 = Fibrosis-4 index, LSM = liver stiffness measurement by VCTE.

Figure 2: Expected patient flow through patient care pathway. Based on estimates available in literature, in the total at risk population of 10.000 participants, we expect 80% to score LSM < 8 kPa (low risk), 17% is expected to score LSM 8 – 12 kPa (indeterminate) and 3% LSM > 12 kPa (advanced fibrosis) upon screening with FibroScan. We assume at least 80% will have LSM ≥ 8 kPa at the re-test. Of those who will be offered liver biopsy, we expect that for 70% MASH diagnosis will be confirmed upon biopsy. In theory this leads to approximately 950 biopsy confirmed diagnoses of MASH. However, considering reluctance for invasive testing and loss to follow-up, we expect to identify approximately 500 confirmed MASH patients in this study, and thus on average 50 per country.





Subject information for participation in medical research

Screening of patients at risk for liver problems due to fat buildup in the liver (GRIPonMASH)

"Global Research Initiative for Patients screening on MASH"

Introduction

Dear Sir/Madam,

With this letter, we would like to ask you to take part in a medical study. Participation is voluntary. You have received this letter because your health care provider found you are at risk of developing liver problems due to fat buildup in your liver and it is possible to be screened for this.

You can read about the medical study in this information sheet. We will explain what it means for you and what the pros and cons of participation are. It is a lot of information. Can you please read the information carefully and decide if you want to take part? If you want to take part, complete the form in Appendix E.

Feel free to ask your questions
You can take your decision based on the information in this information sheet. We also suggest that you do this:

- Ask questions to the health care provider who gave you this information.
- Talk to your partner, family or friends about this study.
- Read the information on: [refer to government information website, if applicable].

1. General information

Julius Clinical has set up this study together with commercial and non-commercial research partners in Europe. This is called a research consortium. Julius Clinical is a research institute located in Zeist, the Netherlands. Below, we always call Julius Clinical the 'sponsor'. Investigators, these can be doctors, research nurses or other hospital staff, carry out the research in different hospitals in collaboration with associated general practitioners. In [country] these are [names of hospitals]. A central Julius Clinical research team oversees the entire study.

This study needs 10.000 subjects from 10 different countries. In [specific county], it is expected that 1000 patients will take part. The Medical Ethics Review Committee [X] has approved this study.

You will have at least one week to decide if you want to take part in the study. After this week you will be approached by the investigator and you can let him/her know your decision.

2. What is the purpose of the study?

This study screens 10.000 patients in 10 countries with an increased risk of liver problems due to fat buildup in the y liver. This way we learn more about how often these liver problems occur and how they can be detected earlier. With the screening program we hope to improve the collaboration between general practitioners and specialized clinics/hospitals by introducing a so-called ‘patient care pathway’. This care path has been established by international scientific associations. The current study is investigating whether less invasive methods to detect liver problems (blood tests and ‘FibroScan’) work just as well as liver biopsy (where a small sample is taken form the liver). We are also trying to find other ways to detect and predict liver problems.

3. What is the background of the study?

More and more people suffer from overweight, obesity and associated diseases like type 2 diabetes mellitus. This is a growing healthcare concern, and it is for example related to an increased risk of cardiovascular diseases. It is also related to metabolic dysfunction-associated steatotic liver disease (MASLD). MASLD is a long-lasting liver condition caused by fat buildup in the liver. MASLD often interferes with the proper functioning of the liver. MASLD can lead to inflammation of the liver (called metabolic dysfunction-associated steatohepatitis; MASH) and scarring of the liver (called fibrosis). Severe MASLD can ultimately cause liver failure (cirrhosis) and can even lead to liver cancer (hepatocellular carcinoma), although the chance of having these severe complications is very small.

The risk of developing MASLD is higher if you have obesity, diabetes, arterial hypertension and 'metabolic syndrome'. It is not yet known exactly how many people in Europe have MASLD. In the early stages MASLD is reversible. That is why it is so important to detect patients with MASLD at an early stage and to start in time with available treatments , like adjusting your lifestyle and in some cases bariatric surgery. Lifestyle changes can for example be, what and how much you eat, and how much you exercise.

4. What happens during the study?

How long will the study take?
Are you taking part in the study? The screening program will be completed within 8 months after enrolling in the study in the hospital. In the third and fifth year after the start, you will be asked again for a short check in the hospital. So the total study duration is 5 years.

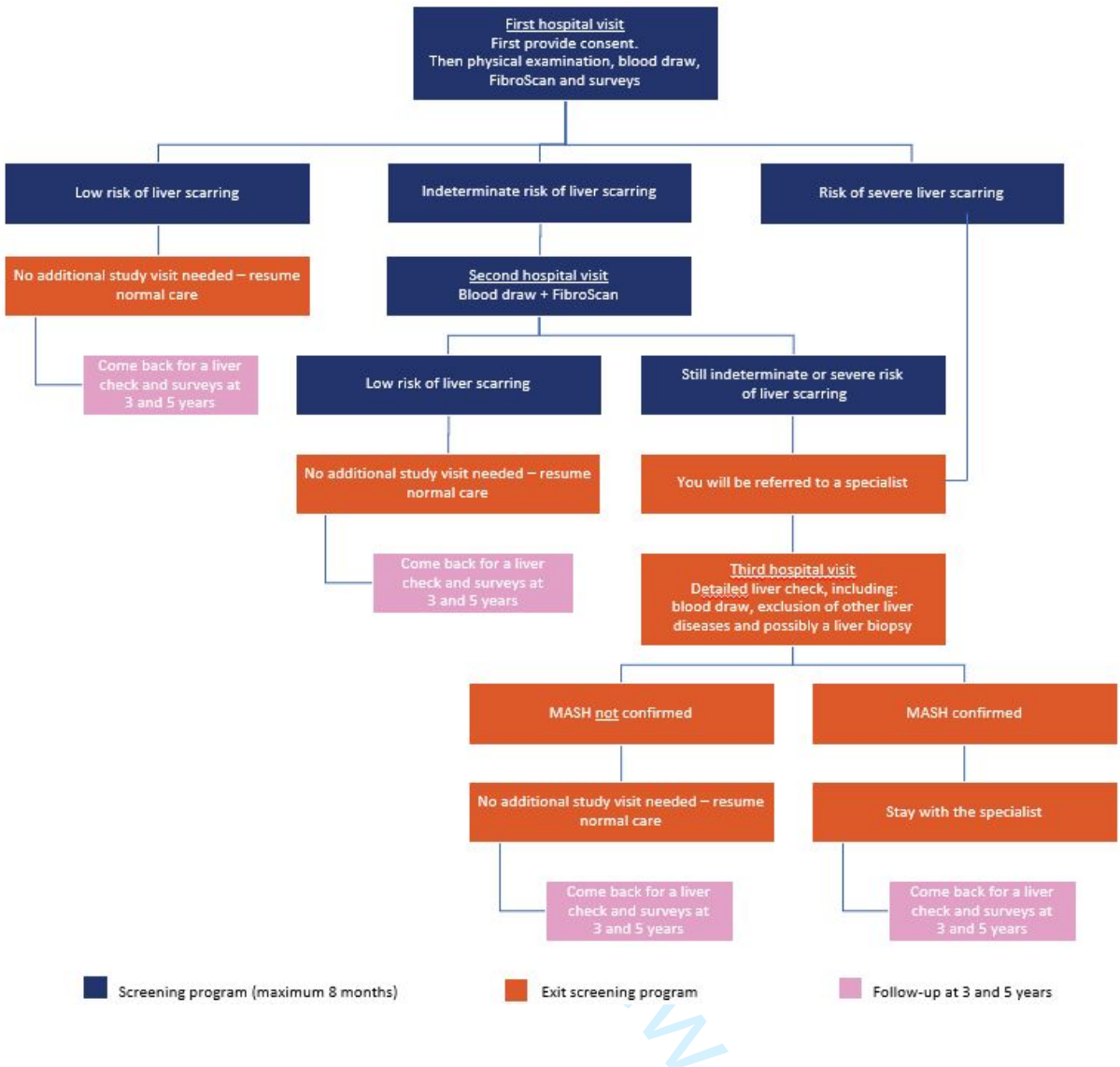
Step 1: are you eligible to take part? This step will take around 10 minutes
First, we want to know if you are eligible to take part. That is the reason that the investigator or general practitioner will do some checks:

- Your medical history.
    - You are only eligible for the screening if you have an increased risk of developing MASLD. In this study defined as: people with obesity, type 2 diabetes, high blood pressure and metabolic syndrome.
    - You cannot have any other liver diseases.
- Excessive alcohol consumption can also damage the liver, so the investigator will ask about your average alcohol consumption.
    - You cannot participate if you consume more than 2-3 units of alcohol per day.

Please note: it is possible that due to other reasons you are not eligible for this study. The investigator will tell you more about this.

Step 2: study and measurements
If you are eligible to participate in the study, you are invited to come to the [name hospital] for further investigation. In the hospital we will first confirm if you are eligible to take part in the study. Then we will check if you have fat buildup or scar tissue on your liver using a kind of ultrasound machine (FibroScan). The outcome of that test determines the further steps within the screening program.

An overview of the screening program (dark blue) and corresponding follow-up checks in the third and fifth year are shown in the figure below:



For the study, you need to visit the [name hospital] at least once. This hospital visit will take approximately one hour. It is important that **you come in fasted**, that means that you are not allowed to eat or drink anything (except water) for at least 8 hours before the hospital visit. The results of the examinations done in the hospital will be sent to you and the doctor or general practitioner who referred you to the study.

We will carry out these checks during the first visit to the hospital:

- Short physical examination. For example, the examiner may measure your blood pressure, weight, length and waist circumference.
- Blood draw. For this, the investigator takes 8 tubes of blood. In total, we will collect approximately 47,5 ml of blood from you. This amount does not cause any problems in adults. For comparison: if you give blood at the blood bank, you will give 500 ml of blood at a time. With the blood test, we measure:
    - Your liver status values
    - Other values related to your health, such as cardiovascular risk factors.
    - If your genes in your DNA are related to a higher risk of MASLD.
    - If there are compounds (such as fats or proteins) in your blood that can predict if you have MASLD or not.
- FibroScan. This is a kind of liver ultrasound that can estimate the amount of fat buildup and scarring of the liver.

- Questionnaires about your diet and lifestyle.
- If the FibroScan provides an indeterminate result, lifestyle recommendations will be provided.
- You may be invited for an interview about your diet, this will extend the visit by 30 minutes. The interview will be conducted by a dietitian or a trained research assistant.

Depending on the results from the first visit, the next visits are scheduled or no additional visits are required:

- *Low risk of liver scarring*: no additional visit needed.
- *Indeterminate risk of liver scarring*: You will be asked to visit the hospital again after 12 weeks for a second FibroScan. We will collect 3 tubes of blood (total approximately 8,5 ml). This will take around 30 minutes.
    - If your liver status improved, no additional visit is needed.
    - If your liver status did not improve, you will be referred to a specialist who will check your liver in more detail. A liver biopsy is performed to be able to definitively diagnose if you have MASH or not. You will be asked to visit the hospital again, and again you need to be fasted. This visit will take around 4 hours as you will have to lay down for a while after the biopsy. We will collect 3 tubes of blood (total approximately 16,5 ml).
- *Severe liver scarring*: you will immediately be referred to a specialist who will check your liver in more detail, according to standard care. We will collect 3 tubes of blood (total approximately 16,5 ml).

Step 3: follow-up check
Three and five years after your first visit, you will be asked to visit the hospital again. At both visits a short physical examination is done. We will collect 3 tubes of blood (total approximately 16,5 ml) and you will be asked to complete the diet and lifestyle questionnaires. At the three year visit we will also do another FibroScan. The results of the FibroScan will again be shared with you and the doctor or general practitioner who referred you to the study.

Appendix C has a list of the measurements we carry out during each visit.

What is the difference with standard care?
This study is an addition to your regular care. The screening program and follow-up checks can be done next to your regular care.

5. What agreements do we make with you?

We want the study to go well. That is why we want to make the following agreements with you:

- You go to every appointment.
- You should contact the investigator in these situations:
    - You are hospitalised or get treatment in a hospital.
    - You suddenly have problems with your health.
    - You no longer want to take part in the study.
    - Your telephone number, address or email address changes.

The next two paragraphs are only applicable for female participants.

Is it OK for you to get pregnant during the study?
*T*his study cannot have any consequences for an unborn child, however we do not know if the

FibroScan does provide a reliable result during pregnancy. Therefore, women who are pregnant cannot participate in this study.

Pregnant after all?
If you do become pregnant during the study, then let investigator know. In the event of a pregnancy, where a FibroScan was still scheduled, you should postpone participation in the study until after the birth in consultation with the investigator.

6. What are the pros and cons if you take part in the study?

Taking part in the study can have pros and cons. We will list them below. Think about this carefully and talk to other people about it.

If you follow the screening program, it will become clear whether you have MASLD or not. If you participate in this research, it does not mean that MASLD will be cured or that you will suffer less from the disease, but your participation will help the investigators to gain more insight into the timely detection of MASLD. You will also help the investigator to gain more insight into the occurrence of MASLD in different countries.

Taking part in the study can have these cons:

- There may be some discomfort from the measurements during the study. For example: taking a blood sample can be a little painful. Or you could get a bruise as a result.
- Taking part in the study will cost you extra time.
- You have to comply with the study agreements.

What are the possible discomforts you may experience with checks or measurements during the study?
Blood sampling is a regular medical procedure with low risks, although in rare cases you may develop bruising after the blood sampling. The FibroScan is a kind of ultrasound and thus has no risks.

In a selected group of patients (see Figure 1) a liver biopsy will be performed. With the collection of liver tissue, there is a risk of bleeding. This bleeding will be cared for immediately.

When you are referred to a specialist to check your liver in more detail, , the specialist will ask for your permission again as these checks are part of standard care. If you participate in the study you give the investigator permission to collect the results of these checks. In case of a liver biopsyyou give permission to collect the results and a bit of your liver tissue.

Possibility of accidental discoveries
It is possible that an accidental discovery is made during the study (for example during the FibroScan, with the blood testing or genetic examination) that is not directly related to the research, but does concern your health. If this happens, your own doctor or specialist will discuss with you what needs to happen next.

You do not wish to participate in the study?
It is up to you to decide if you wish to participate in the study. Do you not wish to participate? You will not be screened for the presence of MASLD and you will continue your health care visits as usual.

7. When does the study end?

The investigator will let you know if there is any new information about the study that is important to you. The investigator will then ask you if you want to continue to take part.

In these situations, the study will stop for you:

- All checks according to the schedule are finished.
- You have become pregnant (in this case the study will be postponed until after the birth).
- You want to stop participating in the study yourself. You can stop at any time. Report this to the investigator immediately. You do not have to explain why you want to stop. You will then exit the screening program and resume your usual health care visits. The investigator will no longer collect information from the moment you indicate that you want to stop participating in the study.
- The investigator thinks it is better for you to stop. The investigator will still invite you for a follow-up check.
- One of the following authorities decides that the study should stop:
    - Julius Clinical
    - the government, or
    - the Medical Ethics Review Committee assessing the study

What happens if you stop participating in the study?
The investigators use the data and body material (the blood samples, and in some cases liver samples) that have been collected up to the moment that you decide to stop participating in the study. If you wish, we will destroy the collected body material. Please let the investigator know. Investigators will always be able to use the data that was collected between the moment you started the study and the moment that you indicated that you want to stop participating in the study.

The entire study ends when all the participants have finished.

8. What happens after the study has ended?

The first analysis is planned within 3 years after the start of the study. You will then receive a report about the most important results of the study.

9. What will be done with your data and body material?

Are you taking part in the study? Then you also give your consent to collect, use and store your data and body material.

What data do we store?
We store these data:

- your gender
- your ethnicity
- your date of birth (month and year only)
- information about your health
- (medical) information that we collect during the study
- Your name
- Your e-mail address
- Your phone number

- Your address
- Results of tests done during the study

What body material do we store?
We store the tubes of blood, and in some case pieces of liver tissue (biopsies). Your DNA can be analysed from the blood and tissue, or other compounds (such as fat and proteins) can be measured. This is further explained in Appendix D.

Why do we collect, use and store your data and body material?
We collect, use and store your data and your body material to answer the questions of this studyand to be able to publish the results.

What do we do with your data?
In this study we work together with commercial and non-commercial partners, which form a research consortium together. The collected data and test results may be shared with these partners to answer the research questions. Also your data will be added to a healthcare platform.

How do we protect your privacy?
To protect your privacy, we give a code to your data and your body material. We only put this code on your data and body material. We keep the key to the code in a safe place in the hospital. When we process your data and body material, we always use only that code. Even in reports and publications about the study, nobody will be able to see that it was about you.

Who can see your data?
Some people can see your name and other personal information without a code. Your referring health care provider and the staff at the hospital will know who you are. The hospital staff executes all physical measurements and collects the data and body material.

The body material is sent to the central storage facility at UMC Utrecht in The Netherlands. The body material will only be referred to by the code, so employees of the central storage facility only know the code. The hospital staff enters the collected data into the central research database. Only coded information is entered, so the central research team at Julius Clinical or other consortium partners only know the code.

Other people who are allowed to see your information without a code, are people checking whether the investigators are carrying out the study properly and reliably. These persons can access your data:

- An auditor and/or monitor who works for the sponsor or is hired by the sponsor.
- National and international supervisory authorities.

These people will keep your information confidential. We ask you to give permission for this access.

For how long do we store your data and body material?
We store your data at the hospital for 15 years. And for 15 years with the sponsor. We store your body materials at the central storage facility at the Utrecht UMC (UMCU Biobank). They will be stored for a maximum of 15 years in order to be able to make new assessments related to this study or in the course of this study. If no longer needed, we will destroy your body material.

Can we use your data and body material for other research?
Your data and your remaining body material may also be important after this study for other medical research on MASLD and related liver problems, such as improving detection methods and developing possible treatment options. For this purpose, remaining body material will be stored at the central

storage facility at the UMC Utrecht for 15 years. Your data will be stored centrally for 15 years. Please indicate in the consent form whether you agree with this. Do you not want to give your consent for this? Then you can still take part in this study. You will enter the same screening program. When a request is made for the use of your data and/ or for the use of your remaining body material, the sponsor will first determine if the request is in line with your consent. If so, the sponsor will request the central storage facility to release the requested remaining body material and/ or will provide the requester with your data. If the other research is of a commercial nature, you as participant will not take share in any possible commercial gains and will not gain a right to the investigational product or medicine.

What will be done with your body material?
Blood samples will be divided in three groups. The first group will be analysed in the hospital. The second and third groups will be shipped to the central storage facility at the UMC Utrecht. The second group of samples will be shipped from the central storage facility to different laboratories and commercial and non-commercial partners in Europe to be analysed. The third group of samples will stay in the storage facility and will be used if additional measurements are needed to answer the research questions or, if you agreed, for other related research.

Liver tissue samples will be sent to the central storage facility at the UMC Utrecht or central analysis directly. Analyses are done at a central laboratory or at commercial and/or non-commercial partners in Europe.

Remaining body material will be stored in the central storage facility and will be used if further or other analysis is needed to answer the research questions or, if you agreed, will be used for other related research.

What happens if there are accidental discoveries?
It is possible that during the study we discover something that is important to your health. In that case, the investigator will contact your referring health care provider. You will then discuss what needs to be done with them.. By signing the form (Appendix E), you give consent to inform your referring health care provider.

Can you take back your consent for the use of your data?
You can withdraw your consent for the use of your data at any time. This applies both to the use in this study and to the use in other medical research. But please note: if you withdraw your consent, and the investigators have already collected data for research, they are still allowed to use this information. The investigators will destroy your body material after you withdraw your consent if you ask them to do so. But if assessments with your body material have been carried out, the investigator can continue to use the results.

Do you want to know more about your privacy?

- Do you want to know more about your rights when processing personal data? Visit [refer to government website if possible].
- Do you have questions about your rights? Or do you have a complaint about the processing of your personal data? Please contact the person who is responsible for processing your personal data. For the present, this is:
    - [name hospital]. See Appendix A for contact details and website.
- If you have any complaints about the processing of your personal data, we recommend that you first discuss them with the research team. You can also contact the Data Protection Officer of [the hospital]. Or you can submit a complaint to the [local Data Protection Authority].

Where can you find more information about the study?
You can find more information about the study on the following website: https://griponmash.eu/
After the study, the website may show a summary of the results of this study.

10. Will you receive compensation if you participate in the study?

Participating in the screening program will not cost you anything. For participating in this study, you will receive an [site-specific: i.e., expense allowance of a maximum of €10,- per visit to the hospital, as a contribution towards travel and parking costs]. If you stop before you have visited the hospital, you will not be reimbursed for expenses.

11. Are you insured during the study?

Insurance has been taken out for everyone who takes part in this study. The insurance pays for damage caused by the study. But not for all damage. You can find more information about this insurance and any exceptions in Appendix B. It also says who you can report damage to.

12. We will inform your general practitioner

The investigator will send your referring health care provider an email to let them know that you are taking part in the study.

As part of the screening program the referring health care provider and research staff of the hospital will exchange information. This following information will be exchanged:

- Results of tests and/or diagnosis or medication use related to the conditions required to join the study.
- The result of the FibroScan (including CAP, LSM, FIB-4 and FAST) will be sent from the hospital to your referring health care provider.
- Results of urine tests related to liver diseases of the past six months (if available) will be sent from your referring health care provider to the research staff at the hospital.

By signing the form (Appendix E), you give consent to the exchange of information as described above.

13. Do you have any questions?

You can ask questions about the study to the research staff at the hospital.

Do you have a complaint? Discuss it with the doctor who is treating you. If you prefer not to do so, please visit [complaints officer/complaints committee of your hospital]. Appendix A tells you where to find this.

14. How do you give consent for the study?

You can first think carefully about this study. Then you tell the investigator if you understand the information and if you want to take part or not. If you want to take part, fill in the consent form that you can find with this information sheet. You and the investigator will both get a signed version of this consent form.

Thank you for your attention.

15. Appendices to this information

A. Contact details <to be adjusted per participating centre>

B. Information about the insurance

C. Schedule of study measurements

D. More information about measurements

E. Consent form

F. Withdrawal from prior consent

A. Contact details hospital and Julius Clinical

If you have any further questions about this study, please contact the research staff at your hospital.

Study centers [text below to be completed for each center]

Center X
Local investigator: [for principal investigator of centre: name, contact details (including phone number) and accessibility]

< if applicable>
[Study nurse/study doctor/nurse specialist]:

Complaints: [service or person with contact details and accessibility]

Data Protection Officer of the institution:

For more information about your rights: [Contact details [including website] of the person(s) responsible for processing personal data]:

<if applicable, to be supplemented with, for example, a coordinating investigator and/or an emergency number/24-hour availability>

Central investigation team at Julius Clinical
Coordinating principal investigators: Assoc. Prof. M. Castro Cabezas and Prof. D.E. Grobbee
Address: Julius Clinical, Broederplein 41-43, 3703 CD Zeist, The Netherlands
Website: https://www.juliusclinical.com/contact/
E: griponmash@juliusclinical.com

B. Information about the insurance

To be added

C. Overview of study measurements

Depending on the outcome of visit 1, either no extra visits are needed, or you will be invited for visit 2 and then possibly visit 3.

	Screening program					Long-term follow up	
Who needs to go to this visit?	All patients		Only if indeterminate risk at 1st FibroScan	Only if risk of severe liver scarring at 1st FibroScan	Only if risk of severe liver scarring at 2nd FibroScan	All patients	
Name of visit	Pre-screening	Visit 1	Visit 2	Visit 3	Visit 4	Follow-up 1	Follow-up 2
Time (from first hospital visit)	- 4 weeks	0	12 weeks	16 weeks	30 weeks	3 years	5 years
Location	GP or hospital	Hospital	Hospital	Hospital	Hospital	Hospital	Hospital
Inclusion/exclusion criteria	x	x					
Demographics (birth date, gender, ethnicity)		x					
Medical history		x					
Medication use		x				x	x
Blood pressure (mm Hg)		x	x			x	x
Height (m)		x					
Weight (kg)		x	x			x	x
Waist circumference (cm)		x	x			x	x
Request recent urine laboratory measurements from health care provider		x					
Questionnaires diet and lifestyle		x				x	x
FibroScan		x	x			x	x
Blood draw		x	x	x	x	x	x
Feedback on FibroScan results		x				x	x
Diet and lifestyle review and lifestyle recommendations (only if indeterminate risk		x					
Optional: interview about diet		x		x	x		

D. More information about study measurements

Genetic and DNA research
We will analyse your genes in your DNA from the blood that will be collected. These genes may be related to the development and/or cure of MASLD. We expect that even more genes will be discovered that play a role in the coming years, so we would like to keep your blood sample for 15 years for further research.

Metabolomic, lipidomic, proteomic and fluxomic research
We will also look at different compounds (such as fats and proteins) that can be found in the blood. For example, we are interested to see if these compounds can predict if you have MASLD or not. Again, we expect that more will be possible in the coming years, and we would like to keep your blood for further research.

FibroScan
The FibroScan is a kind of ultrasound device that works on the basis of pressure waves. Please find a picture of the FibroScan device below. There is no need to take off your clothes for the measurement, only the abdomen must be exposed. The location of the liver is determined and some gel will be applied. For the measurement, the ultrasound probe will be held above the liver and will emit sound waves. The examination is not painful and takes around 10 minutes.

It is important that you arrive at the appointment fasted, meaning you are not allowed to eat or drink anything for some time before the appointment. You will receive more information about the precise rules from your local researchers.

Liver biopsy (optional)
First, the skin above your liver will be numbed. The doctor will then make a small incision in the skin and take a small piece of tissue from the liver through a hollow needle. A liver biopsy is a safe test. In a small number of cases, punctures in the liver may cause bleeding or post-bleeding. That is why you will be asked to lie down for at least 3 hours after the examination so that the doctors can monitor this. You must also arrive fasted at this appointment. We expect that for approximately 20% of the participants a liver biopsy will be advised to determine what liver disease you have and how severe it is.

E. Consent form(s)

Belonging to: *Screening of patients at risk for liver problems due to fat buildup in the liver (GRIPonMASH)*

- I have read the information sheet. I was able to ask questions. My questions have been answered well enough. I had enough time to decide if I wanted to take part.
- I know that taking part is voluntary. I also know that at any time I can decide not to take part in the study. Or to stop taking part. I do not have to explain why.
- I give the investigator consent to inform my referring health care provider that I am taking part in this study.
- I give consent to request information from my referring health care provider about the tests and/or diagnosis or medication use related to conditions to participate in this study and results of urine measurements of the past six months (if available).
- I give consent to give my referring health care provider information about accidental discoveries made during the study that are important for my health.
- I give consent to collect, store, ship and use the results and body material that were obtained during procedures, carried out under standard of care as mentioned in the information letter.
- I give consent to collect, store, ship and use my data and body material. The investigators only do this to answer the question of this study.
- I know that some people will be able to see all of my data to review the study. These people are mentioned in this information sheet. I give consent to let them see my data for this review.
- I know that if I become pregnant, the study appointments are postponed until after the birth.

- Please tick yes or no in the table below.

I give consent to store my data to use for other research, as stated in the information sheet. The data will be stored for this purpose for another 15 years.	Yes ☐	No☐
I give consent to have my (remaining) body material stored and shipped for use in other research, as stated in the information sheet. The body material is stored for this purpose for another 15 years.	Yes ☐	No☐
I give consent to have my DNA (from blood and tissue) stored for use in other research, as stated in the information letter. The DNA is then stored for another 15 years.	Yes ☐	No☐
I give consent to ask me after this study if I want to participate in a follow-up study or drug development research.	Yes ☐	No☐

- I want to take part in this study.

My name is (subject): ……..
Signature: …… Date : __/__/__

I declare that I have fully informed this subject about the study mentioned.

If any information becomes known during the study that could influence the subject's consent, I will let this subject know in good time.

Investigator name (or their representative): ……..
Signature:…… Date: __/__/__

The study subject will receive a complete information sheet, together with a signed version of the consent form.

F. Withdrawal Form for Prior Consent

Belonging to: *Screening of patients at risk for liver problems due to fat buildup in the liver (GRIPon MASH)*

I hereby give notice that I withdraw my participation in GRIPonMASH. This means that no new body material may be taken from me and no more medical data may be collected for GRIPonMASH.

I understand that body material that has been collected from me and has already been processed in the investigation, cannot be recovered or destroyed. Furthermore, I am aware that the medical records used in the study are not recovered or destroyed. This body material and medical data remain encoded and available to the person conducting the investigation.

With regard to the my body materials still stored for GRIPonMASH, I declare that my body material:

O may still be used according to the consent form I previously signed,
O must be destroyed.

My name is (subject): ……
Signature: …… Date : __/__/__

I declare that I have taken note of the withdrawal of consent by the aforementioned patient and as described above.
Institution: ……

Investigator name (or their representative): ……
Signature:…… Date: __/__/__

The investigator will send the signed form to Julius Clinical within 1 week, attn. Study coordinator GRIPonMASH. Julius Clinical will confirm receipt of the form.