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The European NAFLD Registry: A real-world longitudinal cohort study of nonalcoholic fatty liver disease

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ABSTRACT

Non-Alcoholic Fatty Liver Disease (NAFLD), a progressive liver disease that is closely associated with obesity, type 2 diabetes, hypertension and dyslipidaemia, represents an increasing global public health challenge. There is significant variability in the disease course: the majority exhibit only fat accumulation in the liver but a significant minority develop a necroinflammatory form of the disease (non-alcoholic steatohepatitis, NASH) that may progress to cirrhosis and hepatocellular carcinoma. At present our understanding of pathogenesis, disease natural history and long-term outcomes remain incomplete. There is a need for large, well characterised patient cohorts that may be used to address these knowledge gaps and to support the development of better biomarkers and novel therapies.

The European NAFLD Registry is an international, prospectively recruited observational cohort study that aims to establish a large, highly-phenotyped patient cohort and linked bioresource. Here we describe the infrastructure, data management and monitoring plans, and the standard operating procedures implemented to ensure the timely and systematic collection of high-quality data and samples. Already recruiting subjects at secondary/tertiary care centres across Europe, the Registry is supporting the European Union IMI2-funded LITMUS 'Liver Investigation: Testing Marker Utility in Steatohepatitis' consortium, which is a major international effort to robustly validate biomarkers that diagnose, risk stratify and/or monitor NAFLD progression and liver fibrosis stage. The European NAFLD Registry has the demonstrable capacity to support research and biomarker development at scale and pace.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide and represents a major global public health challenge. It is characterised by the increased accumulation of hepatic fat (> 5%) and is closely linked with the presence of the metabolic syndrome and its components: obesity, type 2 diabetes mellitus, hypertension and dyslipidaemia. [1,2] The exclusion of other causes of

Fig. 1. Map of recruiting sites into the European NAFLD registry.
The European NAFLD Registry is actively recruiting at sites across Europe using a 'hub-and-spoke' model. National lead sites (red), recruiting centres (blue). Additional recruitment is also taking place at selected sites in USA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fat accumulation such as excessive alcohol consumption is traditionally part of the disease definition although a recently proposed revised nomenclature, metabolic-dysfunction associated fatty liver disease (MAFLD), places emphasis on the pre-eminence of metabolic-dysfunction. [3] NAFLD is a spectrum of progressive liver disease comprising steatosis (non-alcoholic fatty liver, NAFL), in which excessive hepatic fat is present, and non-alcoholic steatohepatitis (NASH), a necroinflammatory form of the condition marked by histological inflammation and hepatocyte ballooning that leads to progressive liver fibrosis. NAFLD is a cause of significant morbidity and mortality; left untreated, fibrosis may progress to cirrhosis and can result in end-stage liver disease or hepatocellular carcinoma (HCC). [4–6]

The prevalence estimates of NAFLD vary widely according to the modality used to detect NAFLD and the geographical area. A recent meta-analysis estimated the global prevalence of NAFLD to be 25% of adults, with the highest estimates in the Middle East and South America (32% and 31%, respectively) and the lowest estimates in Africa (14%); the estimates for Asia, the USA, and Europe were 27%, 24% and 23%, respectively. [2,7] Modelling studies predict a steady increase in disease incidence in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States that is accompanied by an increase in liver transplantation, HCC and mortality from liver and non-liver-related causes. [8] The associated economic burden is substantial and, without a concerted public health response, will continue to increase as more people are affected. [9–11]

NAFLD is characterised by substantial inter-patient variability in terms of severity and rate of progression. [12] Despite there being a large at-risk population, only a minority experience significant morbidity. The factors that determine transition from NAFL to NASH, and subsequent progression of fibrosis to cirrhosis are incompletely understood. Thus, there are key knowledge gaps, in particular limited clarity on modifiers of disease natural history and an incomplete understanding of disease pathophysiology. These issues contribute to the lack of tractable non-invasive biomarkers of disease severity that hinder the diagnosis, risk stratification and monitoring of patients, and the absence of approved pharmacological therapies.

Although many people have NAFLD, few are sufficiently well characterised for their data to be tractable in research. This is because histopathological examination of liver tissue is required to differentiate NAFL from NASH, grade steatohepatitis activity and accurately stage fibrosis. [5] However, liver biopsy is not widely utilised outside specialist centres as it is resource-intensive and carries a small but appreciable risk of complications. Therefore, a broad collaborative effort is required to pool sufficient data from comprehensively phenotyped patients at specialist centres to assemble an adequately sized cohort that may be leveraged to support research. The European NAFLD Registry (ENR) is an innovative, international patient registry with an associated bioresource that is assembling a large, well-characterised patient cohort specifically to support translational and discovery science.

Table 1
Current recruitment sites by country.

Country	National lead investigator	Site name	Site PI
UK	Quentin Anstee	Freeman Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust	Quentin Anstee
		Barts Health NHS Trust, London	William Alazawi
		Hull University Teaching Hospitals NHS Foundation Trust	Lynsey Corless
		Oxford University Hospitals NHS Foundation Trust	Jeremy Cobbold
		Portsmouth Hospitals NHS trust	Joanna Dowman
		Cambridge University Hospitals NHS Foundation Trust	Mike Allison
		University Hospitals Birmingham NHS Foundation Trust	Philip Newsome
		Nottingham University Hospitals NHS Trust	Guruprasad Aithal
		University Hospitals Plymouth NHS Trust	David Sheridan
		Queen Elizabeth Hospital, Gateshead	Dina Mansour
		City Hospitals Sunderland, Sunderland	Harriet Mitchison
		St Georges Hospital, London	Daniel Forton
		Leeds Teaching Hospitals NHS Trust	Richard Parker
France	Vlad Ratziu	Assistance Publique – Hôpitaux de Paris	Vlad Ratziu
		Angers University Hospital	Jerome Boursier
Italy	Elisabetta Bugianesi	University of Torino	Elisabetta Bugianesi
		University of Ancona	Gianluca Svegliati- Baroni
		Università Cattolica del Sacro Cuore of Rome	Luca Miele
		University of Palermo	Salvatore Petta
		University of Milano	Luca Valenti
Germany	Andreas Geier Jörn Schattenberg	University of Wurzburg	Andreas Geier
		University Medical Centre Mainz	Jörn Schattenberg
		Charité University Hospital, Berlin	Münevver Demir
		Hannover Medical School	Heike Bantel
		Uniklinik RWTH Aachen	Christian Trautwein
Spain	Manuel Romero-Gomez	Andalucia Health Service, Seville	Manuel Romero-Gomez
		Puerta de Hierro Hospital, Majadahonda	Jose Luis Calleja
		Vall d'Hebron Hospital, Barcelona	Salvador Augustin
		Biodonostia Research Institute-Donostia University Hospital, San Sebastián	Jesús Banales & Rocio Aller
		University Hospital Valladolid, Valladolid	Rocio Aller
		Marques de Valdecilla Hospital, Santander	Javier Crespo
USA	Stephen Harrison	Pinnacle Clinical Research, San Antonio, Texas	Stephen Harrison
Portugal	Helena Cortez Pinto	University of Lisbon	Helena Cortez Pinto
Belgium	Sven Francque	University Hospital Antwerp	Sven Franque
Netherlands	Adriaan Holleboom	Amsterdam University Medical Centre	Adriaan Holleboom & Ulrich Beuers
Sweden	Mattias Ekstedt	Linköping University	Mattias Ekstedt
		Karolinska Institutet, Stockholm	Hannes Hagström
Greece	George Papatheodoridis	National and Kapodistrian University, Athens	George Papatheodoridis
Finland	Hannele Yki- Jarvinen	Helsinki University	Hannele Yki- Jarvinen
Switzerland	Jean-Francois Dufour	Bern University	Jean-Francois Dufour

2. Methods

2.1. Overview

The European NAFLD Registry is a prospectively recruited, international observational study to assemble a cohort of highly-phenotyped patients across the full spectrum of NAFLD that will facilitate *cross-sectional* and *longitudinal* analyses (clinicaltrials.gov registration NCT04442334) [13]. The comprehensive dataset includes clinical information, liver histopathology and imaging data derived from healthcare records during routine medical care and is supplemented by lifestyle and quality of life data, as well as biological sample collection.

The European NAFLD Registry infrastructure hosts a uniform dataset of patient level data that is split into two distinct sub-sections:

i. *The 'Metacohort'*: comprises prospectively recruited NAFLD cases that consented to participate during recruitment for European studies into NAFLD pathogenesis conducted from 2010 to December 2017 (i.e. the FP7-FLIP, H2020-EPoS and HEPAmet cohorts) [14–16], plus cases with compatible consent and data collection processes derived from previous investigator-led single-centre cohorts. Data from these cohorts have been integrated into a single dataset whilst maintaining data quality and retaining knowledge of the source.
ii. *The 'LITMUS Study Cohort'*: comprises prospectively recruited NAFLD cases acquired since 1st January 2018 as part of the IMI2-funded LITMUS project [17]. Recruitment is ongoing across Europe according to a common Master Study Protocol with a comprehensive data monitoring process and standard operating procedures for sample handling implemented. Recruitment is currently active at > 25 centres in 13 countries (Fig. 1 and Table 1.)

Patients with that are enrolled into the European NAFLD Registry will be followed-up for up to 10-years to allow assessment of long-term outcomes.

2.2. Objectives

The primary objective of the European NAFLD Registry observational study is to assemble a 'real-world' cohort of well-characterised patients across the full spectrum of NAFLD and to collect associated clinical information, biological samples and imaging data for *cross-sectional* and *longitudinal* analyses to support research into disease natural history.

Key secondary objectives are to support research addressing the pathophysiology of NAFLD using a range of state-of-the-art scientific techniques with integrated bioinformatics. Additional secondary measures include the study of dietary habits, lifestyle/activity factors and symptom burden.

To characterise and integrate key intrinsic factors multiple 'omics' approaches (genetic, epigenetic, transcriptomic, metabolomic, proteomic and metagenomic) will be applied to understand inter-individual variation in severity of hepatic injury, serum and hepatic 'omic' profiles and their interaction with environmental (behavioural/dietary/lifestyle) factors that determine how a patient feels, how the disease progresses over time and long term outcomes. We envisage these activities will lead to a substantial and definitive atlas of pathophysiological variation across the spectrum of progressive NAFLD, identify predictors of long-term outcome and support identification of novel biomarkers and therapeutic targets.

In terms of application, the present focus of activity for the European NAFLD Registry is to support the work of the European Union IMI2-funded LITMUS 'Liver Investigation: Testing Marker Utility in Steatohepatitis' consortium (https://www.imi.europa.eu/projects-results/project-factsheets/litmus). This ambitious project brings together clinicians and scientists from prominent academic centres across Europe with companies from the European Federation of Pharmaceutical Industries and Associations (EFPIA). Their common goals are developing and validating biomarkers for testing NAFLD and seeks to establish a defined set of biomarkers that singly or in combination, enable detection and monitoring of disease progression to/

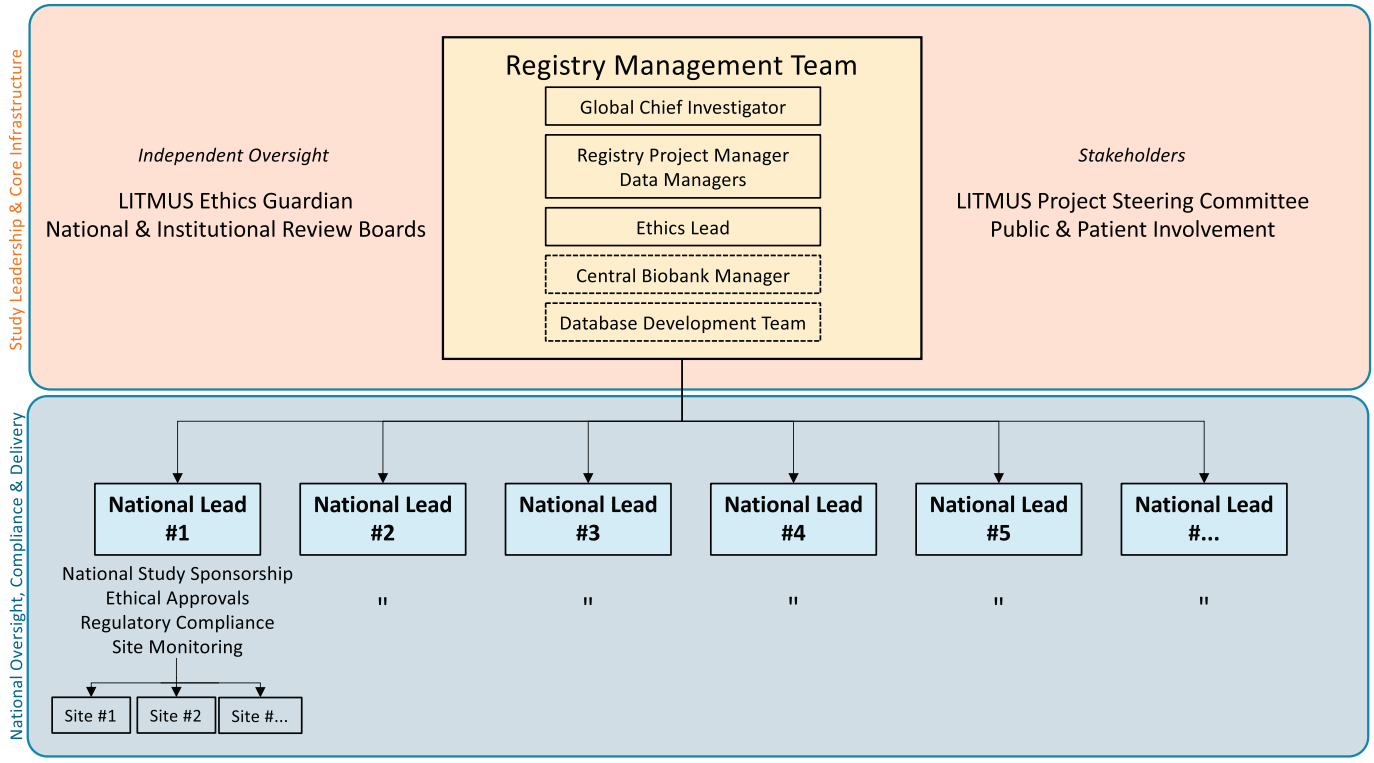

Fig. 2. Overview of the European NAFLD registry governance structure.

regression from NAFL through NASH to fibrosis and cirrhosis. Through the Registry, research is powered to provide the required clarity on biomarker analytical and clinical validity at scale and pace, generating the requisite level of high-quality data to support biomarker validation and evidence needed for regulatory qualification under the joint EMA Committee for Medicinal Products for Human Use (CHMP) and the US Food and Drug Administration (FDA) processes.

2.3. Organisation & oversight

The European NAFLD Registry operates across multiple territories and so a comprehensive organisational and oversight structure has been established (Fig. 2) that defines lines of responsibility as well as project management, data management, data monitoring and sample handling processes. Oversight and recruitment into the Registry are structured according to three tiers of geographical hierarchy: central leadership and coordination, national oversight and site level delivery.

Coordination of the study, including establishing the European NAFLD Registry Master Protocol, defining standard operating procedures, management of the web-based eCRF database, controlling data access and biological sample use are managed centrally by the Registry Management Team. This group comprises the Chief Investigator, Study Managers, Data Managers, Ethics Lead and representatives from the Registry Central Biobank. However, the conduct of the study according to these centrally defined processes, including responsibility for study sponsorship, regulatory compliance, gaining the necessary national and/or local ethical approvals for recruitment and site data-monitoring as centrally defined is devolved to a budget-holding National Lead Investigator in each country (Table 1). These senior investigators are experienced clinical trialists with extensive knowledge of the national research frameworks in which they operate. Thus, the Registry is able to tailor its activities to varying national research ecosystems whist ensuring robust data collection in compliance with the *International Conference on Harmonisation - Good Clinical Practice (ICH-GCP)* requirements [18].

2.3.1. Ethical practice & regulatory compliance

All subject recruitment and informed consent processes at recruitment centres are conducted in compliance with nationally accepted practice in the respective territory and in accordance with the *World Medical Association Declaration of Helsinki 2018*, the *Charter of Fundamental Rights of the European Union (2000/C 364/01)*, and the principles defined by the *Belmont Report*. Data is collected and processed in accordance with the applicable *General Data Protection Regulation (EU) 2016/679 (GDPR)* legislation.

For the 'LITMUS Study', a named National Lead Investigator oversees the conduct of recruitment at sites in their respective country (Table 1). An Ethics Lead curates the central LITMUS Study Trial Master File (L-TMF) to ensure all necessary ethical approvals are in place for each territory and reports annually to an independent ethics guardian. Supplementary Material 1 provides details of the associated ethical approvals by country.

2.3.2. Database

Personal data including clinical data are collected, and such data are protected in accordance with the European *General Data Protection Regulation (GDPR)*. Data is held within the Registry in a pseudoanonymised (linked-anonymised) form to avoid personally identifiable data transfer or processing outside a subject's usual clinical care team at the recruiting site.

A purpose-built MySQL relational database has been developed to facilitate data capture during recruitment into the Registry. The web-accessible secure front-end database comprises 14 related electronic clinical record form (eCRF) data-tables and contains around 1200 data fields, equating to approximately 1–2 megabytes of data per subject and is designed to fulfil the regulatory requirements for record keeping [19]. Data are held in a Clinical Data Interchange Standards Consortium (CDISC, www.cdisc.org) compliant format to aid information system interoperability for future collaborative projects. The database holding the project user records and collected records from sites is hosted on a multi-machine replication cluster with multiple physical servers. In the event of machine failure or catastrophic data loss, one of the remaining database servers would be promoted to become the master server for the European NAFLD Registry application. Database records are archived to a separate physical server three times daily, once per day for the previous 31 days and once per month for the previous 12 months. In addition, an archive copy of the data is retained on Newcastle University's Research Data Warehouse according to local information security policy. This ensures a copy of the data is available for up to ten-years after the last date of access. Network access to the database is controlled by specific role at the individual level.

Table 2
Inclusion and Exclusion criteria.

Criteria	
Inclusion	1. Age ≥ 18 years and able to give informed consent 2. Clinically suspected NAFLD based on the following: A. Historical liver biopsy providing histological evidence of NAFLD or, B. Biochemical and/or radiological evidence of NAFLD in patients undergoing liver biopsy or, C. Radiological evidence of cirrhosis (in absence of an alternative aetiology) plus ≥2 features of the 'metabolic syndrome' i. Increased waist circumference by ethnically adjusted criteria (e.g. Europid male/female ≥94 cm/80 cm or overweight/obese (BMI ≥ 25 kg/m^2); ii. Raised fasting glucose ≥100 mg/dL [5.6 mmol/L], HbA1c ≥48 mmol/mol [6.5%] or previously diagnosed insulin resistance/ type 2 diabetes mellitus, or on treatment; iii. Dyslipidaemia (fasting triglyceride level ≥ 150 mg/dL [1.7 mmol/l]; or fasting high density lipoprotein < 40 mg/dL [1.03 mmol/l] in males and < 50 mg/dL [1.29 mmol/l] in females, or on treatment; iv. Hypertension (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg), or on treatment.
Exclusion	1. Refusal or inability to give informed consent 2. Average alcohol consumption greater than 21/14 units per week (30/20 g/alcohol/day) (males/females) in the preceding 6 months and no history of sustained excessive alcohol consumption of alcohol in the past five years. 3. History or presence of Type 1 diabetes mellitus 4. Presence of any other form of chronic liver disease 5. Recent (within 12 months) or concomitant use of agents known to cause hepatic steatosis (long-term systemic corticosteroids [> 10 days], amiodarone, methotrexate, tamoxifen, tetracycline, high dose oestrogens, valproic acid) 6. Any contraindication to liver biopsy 7. Recent (within 3 months) change in dose/regimen or introduction of Vitamin E (at a dose of ≥400 IU/day), betaine, s-adenosyl methionine, ursodeoxycholic acid, silymarin or pentoxifylline 8. Non-local language speaker/unable to access interpreter 9. Patients not meeting the inclusion criteria or judged by the investigator to be unsuitable for inclusion into the study

2.3.3. Biorepository

Biological samples are physically stored in the secure, ISO 9001 certified and ISO 17025 accredited biobank at the state-of-the-art facilities of the Integrated BioBank of Luxembourg (IBBL) Institute.

2.4. Inclusion/exclusion criteria

The study population is patients aged ≥18 years with risk factors for NAFLD that will be recruited in hepatology clinics and/or bariatric surgery units primarily distributed at centres across Europe. Patients are invited to participate in both the cross-sectional and longitudinal aspects of the study but may drop-out of the latter at any point if they wish. Two main sources of patients are targeted:

i. Patients investigated for suspected NAFLD in hepatology clinics, e.g. referred for the investigation of abnormal liver biochemistry tests noted in primary care.
ii. Patients seen in bariatric surgery units for planned surgical weight loss treatment for morbid obesity.

Inclusion and exclusion criteria are presented in Table 2. In summary, for non-cirrhotic patients, confirmation of the diagnosis of NAFLD must be made histologically, whereas patients with radiological evidence of cirrhosis in the absence of an alternative aetiology may be recruited if at least two features indicative of the metabolic syndrome are present. Secondary causes of steatosis, including high alcohol consumption, and other coexisting liver diseases are exclusionary.

2.5. Study procedures

Study processes have been designed to minimise the burden of participation on patients. As much as possible, data and samples are collected alongside scheduled routine-care clinical attendance. Following signed informed consent, patients are assigned a unique 'study participant identification code' (SPIC). This ensures data are held in the Registry in a linked-anonymised form to preserve patient confidentiality. An Investigator Handbook has been developed to assist study staff at sites in application of standardised processing methods to avoid any centre-related preanalytical bias (Supplementary Material 2).

2.5.1. Data collection

A comprehensive dataset is collected from the medical notes at the 'baseline' enrolment clinical attendance (usually coincident with a liver biopsy). This detailed dataset includes demographics, anthropometrics, a range of clinical (co-morbidities, current medication), laboratory (clinical haematology, biochemistry, immunology), histopathological and radiological data (Table 3).

Diagnostic imaging data are collected for all patients undergoing clinically indicted investigations. These include routine diagnostic ultrasound and Fibroscan™ (vibration-controlled transient elastography [VCTE]) as well as any other modalities that may be used as part of local practice at recruiting sites. In addition, selected patients are invited to opt-in to participate in a nested imaging study in which MRI-PDFF, MR LiverMultiScan™, deMILI, and MR Apparent Diffusion Coefficient, as well as elastography (acoustic radiation force impulse [ARFI]; and magnetic-resonance elastography [MRE]) are captured outwith standard care.

In addition, the following lifestyle and symptom burden questionnaires are collected:

i. *Patient-reported health-related quality of life (HRQOL):*

- Chronic Liver Disease Questionnaire for NAFLD NASH (CLDQ NAFLD-NASH) [20,21],
- EQ-5D-5L Health [22],
- NASH-CHECK [23].

ii. *Dietary questionnaires:*

- Audit C [24],
- Mediterranean Diet Score [25].

iii. *Lifestyle (exercise/sedentary behaviour assessment):*

- International Physical Activity Questionnaire (IPAQ) [26].

Thereafter, 'follow-up event' data are collected annually at scheduled out-patient clinic attendances. In addition, data collection may be triggered if the patient undergoes a subsequent clinically indicated liver biopsy or if a significant 'clinical event' occurs. Target events are listed in Table 4 and focus particularly on death, major adverse cardiovascular events (MACE), hepatic events (progression to cirrhosis, hepatic decompensation and/or HCC) and extra-hepatic malignancy.

Where healthcare systems permit (for example, the UK's National Health Service "NHS Digital" platform), patients are asked to allow their national health records to be flagged to facilitate capture of long-term outcome events if they are lost to follow up at the recruitment site.

Table 3
Summary of clinical data collected at enrolment and follow-up events.

Categories of data
Basic data • Date of Birth and Age at event • Sex at Birth • Self-reported ethnicity • Anthropometrics: o Height (cm) o Weight (kg) o Waist circumference (cm) o Hip circumference (cm)
Medical history • Relevant comorbidities, including: o Hypertension, Dyslipidaemia, Type 2 Diabetes o Obstructive sleep apnoea o Malignancy o Cardiovascular disease/Stroke o Other relevant • Current/Recent medication (including over-the-counter and traditional/herbal remedies) • Participation in any therapeutic clinical trials
Results of clinical investigations • Results of local histopathological assessments of liver biopsy. • Results of routine Haematology, Clinical Biochemistry, Immunology, and Virology investigations (all with date of sample collection) o The following should be collected within ± 30 days of liver biopsy and in a fasting condition: ▪ Haematology (FBC, Clotting) ▪ Clinical Biochemistry (U&E, LFTs [Alb, Bili, ALP, ALT, AST, gGT], Ferritin/Transferrin saturation, HBA1C, Glucose, Insulin, C-peptide, TSH, Lipid profile) ▪ Biomarkers (if available, e.g. CK18, ELF) o The following are not time limited: ▪ Viral serology (HBV, HCV) ▪ Auto-antibody screen, Immunoglobulins (IgG, IgA, IgM) ▪ A1AT, Copper/Caeruloplasmin • Ultrasound imaging • Ultrasound based Elastography (e.g. Fibroscan [Transient Elastography/CAP], ARFI, SuperSonic Imaging)
Lifestyle • Average alcohol intake (units/week) for last 6 months as a quantitative variable • Alcohol intake – any history of alcohol excess? • Smoker – Yes/No/Ex • Tea/Coffee consumption – cups/day • Social factors including employment and education status
Family history • Limited family medical history (first degree relatives)

Table 4
Key clinically significant events targeted during follow-up.

Event category	
Death*	
Major Adverse Cardiovascular Events	1. Non-fatal Stroke (CVA) 2. Non-fatal myocardial infarction ('STEMI' or 'Non-STEMI') 3. Coronary revascularisation (angioplasty, CABG) 4. Hospitalisation for heart failure
Hepatic	1. Diagnosis of cirrhosis 2. Diagnosis of varices at endoscopy 3. Variceal haemorrhage 4. Hepatic decompensation (jaundice, ascites, encephalopathy – including commencement of treatment for encephalopathy or ascites) 5. Hepatocellular carcinoma 6. Liver transplantation
Other	1. Diagnosis of extra-hepatic malignancy 2. Emergency hospitalisation

* In the event of death, cause of death is recorded.

In addition, if a patient were to move their regular care provider from one participating centre to another, the patient can transfer their unique SPIC to the new participating site so that the new site can continue to update the patient's registry record.

2.5.2. Sample Collection & Processing

Following informed consent and study code (SPIC) assignment, patients provide biological samples for research use at enrolment (baseline). Blood samples are drawn in the fasted state as close to the date of the liver biopsy as possible (± 30 days). Collected biological samples include: whole blood for serum and plasma isolation, for genomic DNA extraction, then for circulating miRNA and circulating cfDNA extraction from plasma; stool for microbial DNA extraction; urine; and liver tissue (formalin-fixed, paraffin embedded [FFPE] and snap-frozen samples), if the patient is undergoing a clinically indicated liver biopsy. Samples to be collected are summarised in Fig. 3, which provides an overview of how samples are processed and transferred to the central biobank facility. Faecal microbiome analyses are known to be significantly impacted by different DNA extraction methods, therefore all stool DNA extractions are centrally performed at the IBBL with a

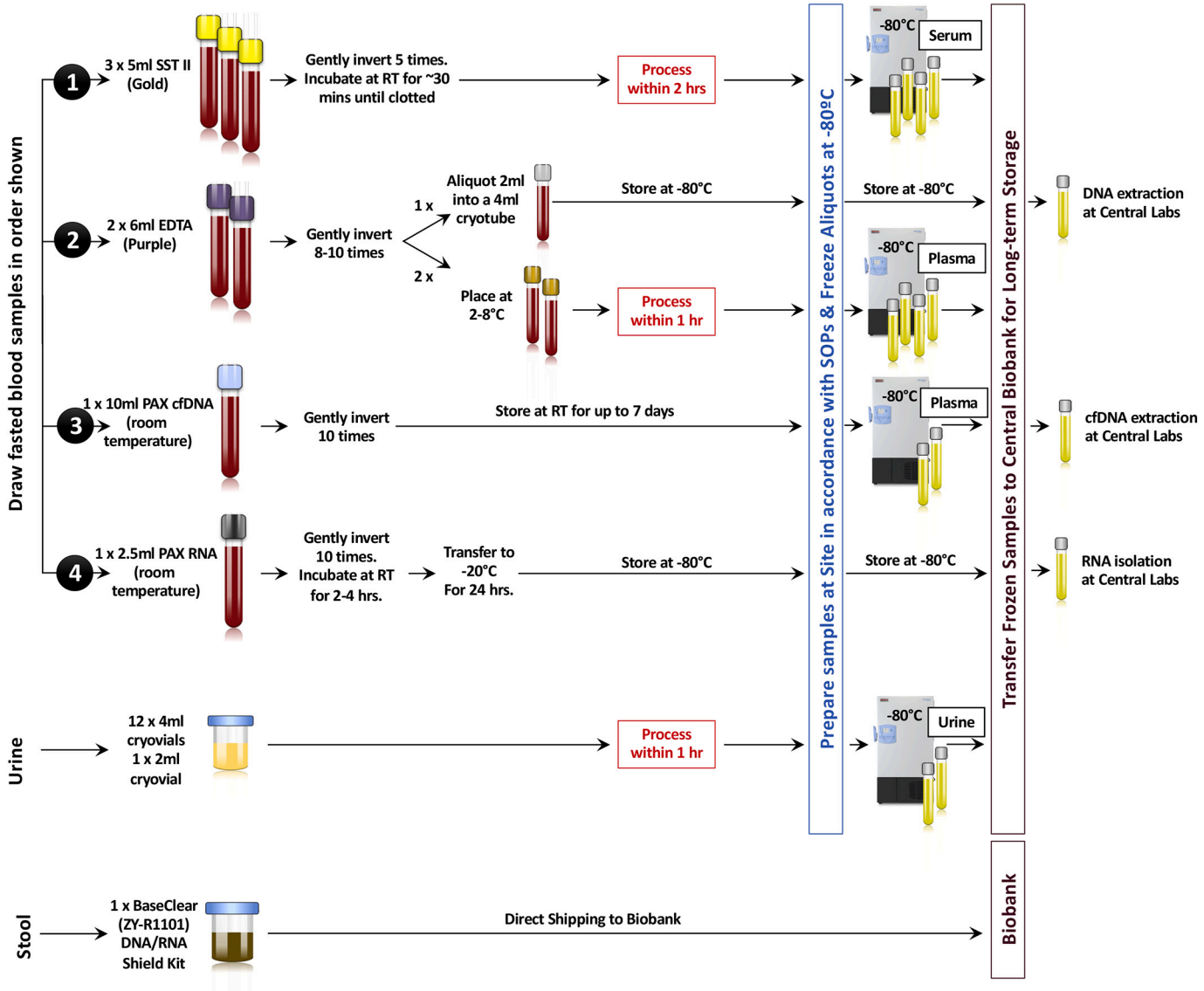

Fig. 3. Summary of biological sample collection processing.
At enrolment (Baseline) and at subsequent annual follow-up visits, or if a clinically significant event occurs, a range of samples will be collected from patients participating in The European NAFLD Registry. These will be processed according to defined SOPs and shipped to the Central Biobank facility for storage.

validated method [27]. At subsequent annual routine hospital attendances or major clinical events, patients are asked to provide follow-up biological samples for longitudinal studies. To ensure high quality samples are consistently collected across all sites and to minimise pre-analytical variation, standard operating procedures have been established and are fully described in the Investigator Handbook (Supplementary Material 2).

2.5.3. Central histological interpretation & digitisation of liver biopsies

Histopathological assessment of liver biopsy remains the widely accepted, albeit imperfect, reference standard for assessing severity of liver disease [28]. As well as issues with sampling error [29], there remain concerns regarding potential interobserver variability in assessment. To address these issues, the European NAFLD Registry has adopted a number of measures to ensure data quality:

i. *Histology Processing at a Central Laboratory*. To minimise variation in slide staining between laboratories, biopsies are fixed in 10% buffered formalin, embedded in paraffin and cut into 3–4 μm sections at recruitment sites. Four unstained slides are then shipped to the Central Biobank for staining, digitisation and storage. At IBBL, slides are stained with haematoxylin and eosin (H + E) and Masson's trichome for collagen according to established standard operating procedures. Then high-resolution digitised images are captured using the CaloPix® platform (Tribvn Healthcare, France).
ii. *Semiquantitative histological scoring systems* are used to standardise reading of biopsies. Grade of steatosis/steatohepatitis are reported according to both the well validated NASH Clinical Research Network (CRN) "NAFLD Activity Score" (NAS) and the FLIP "Steatosis – Activity – Fibrosis" (SAF) systems [30,31]. Whilst these scoring systems differ in important ways, all measure four key histological characteristics: grade of steatosis, severity of hepatocellular injury (hepatocyte ballooning) and lobular inflammation, and stage of fibrosis (summarised in Table 5). Fibrosis stage is recorded using both the NASH CRN/SAF 5-tier (F0-4) and the EPoS 7-tier (F0-6) fibrosis staging systems (including sub-staging of cirrhosis into stages 6a, 6b and 6c according to modified Laennec staging) [32,33]. In addition to histological scoring of these standard features, pathologists also assess the intensity of portal inflammation, the presence of Mallory-Denk bodies and determine a broad diagnostic category (i.e. normal, NAFL, definite NASH, fibrosis/cirrhosis without steatosis consistent/not consistent with NAFLD). All data are captured using a standardised reporting proforma.
iii. *The Registry Histopathology Group (RHG)* comprises ten expert hepatopathologists (Table 6) who participate in a face-to-face harmonisation meetings to align on histological criteria and have demonstrated close interobserver concordance [31]. Pairs of hepatopathologists from this group independently double-score liver biopsies with reference to a digital atlas of NAFLD histology that has been prepared by the group leaders (Supplementary Material 3 and via the European Society of Pathology website: https://tinyurl.com/LITMUS-Histology). If the two scores within a pair are found to be discrepant (by ≥2 points for steatosis or inflammation, and ≥ 1 for ballooning or fibrosis) the biopsy is referred to a consensus adjudication panel. To further ensure data quality, the adjudication panel reviews a random sample of biopsies for quality control. An administrator assigns the liver biopsies to the pathologist pairs, completes the final report for each case based on the agreed scores and uploads the histological data in the NAFLD Registry.

Although semiquantitative scoring systems are used in many clinical trials [34–38], it is recognised that scoring systems provide a non-linear, semiquantitative or categorical assessment of disease that may limit precision and granularity of data, particularly in the context of subtle changes over time or at the boundary between two categories, where misclassification may occur. To supplement these efforts, the European NAFLD Registry also captures data from a number of novel automated quantitative assessment measures based on digitised biopsy images and second harmonic generation/two-photon excitation microscopy (Genesis200®, HistoIndex, Singapore), leveraging machine learning and artificial intelligence approaches to add further value to

Table 5
Comparison of the SAF Score and the NAFLD Clinical Research Network Score for the histological grading and staging of NAFLD.

SAF score [42]			NASH CRN NAFLD activity score [30]		
Histological feature	Category definition		Histological feature	Category definition	
Steatosis†	0	< 5%	Steatosis‡	0	< 5%
	1	5–33%		1	5–33%
	2	34–66%		2	34–66%
	3	> 66%		3	> 66%
(S) Steatosis Score 0–3			PLUS		
Hepatocyte Ballooning	0	None	Hepatocyte Ballooning	0	None
	1	Clusters of hepatocytes with rounded shape and pale and/or reticulated		1	Few
	2	cytoplasm		2	Many
		Same as grade 1 with enlarged hepatocytes (> 2× normal size)			
PLUS			PLUS		
Inflammation	0	None	Inflammation	0	None
	1	≤ 2 foci per 20× field		1	≤2 foci per 20× field
	2	> 2 foci per 20× field		2	2–4 foci per 20× field
				3	> 4 foci per 20× field
(A) Total = Activity Score 0–4*			(NAS) Total = NAFLD Activity Score 0–8		
Fibrosis	0	No significant fibrosis	Fibrosis	0	No significant fibrosis
	1a	Zone 3 mild perisinusoidal fibrosis		1a	Zone 3 mild perisinusoidal fibrosis
	1b	Zone 3 moderate perisinusoidal fibrosis		1b	Zone 3 moderate perisinusoidal fibrosis
	1c	Periportal/portal fibrosis only		1c	Periportal/portal fibrosis only
	2	Zone 3 plus portal/periportal fibrosis		2	Zone 3 plus portal/periportal fibrosis
	3	Bridging fibrosis		3	Bridging fibrosis
	4	Cirrhosis		4	Cirrhosis
(F) Fibrosis Score 0–4			Fibrosis Score 0–4		

† Percentage of parenchymal involvement by steatosis.
‡ Percentage of hepatocytes containing large and/or medium-sized intracytoplasmic lipid droplets.
* SAF A_1 (A = 1): mild activity; A_2 (A = 2): moderate activity; A_3 & A_4 (A > 2): severe activity. Table adapted from [28,43].

Table 6
Organisation of the Registry Histopathology Group: pairing and adjudication panel.

Pathologist pair number	Pathologist	Institution
1	Johanna Arola	University of Helsinki, Helsinki, Finland
	Pierre Bedossa	LiverPat, Paris, France; Translational & Clinical Research Institute, Newcastle University, UK
2	Susan Davies	Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
	Dina Tiniakos	Translational & Clinical Research Institute, Newcastle University, UK; Medical School, National and Kapodistrian University of Athens, Greece
3	Stefan Hübscher	Queen Elizabeth Hospital, University of Birmingham, UK
	Valerie Paradis	Hôpital Beaujon, Université Paris-Diderot, Paris, France
4	Beate Straub	Institute of Pathology, University Medical Centre Mainz, Mainz, Germany
	Joanne Verheij	Amsterdam University Medical Centre, The Netherlands
5	Alastair Burt	Translational & Clinical Research Institute, Newcastle University, UK
	Ann Driessen	University of Antwerp, Antwerp, Belgium
Adjudication Panel	Dina Tiniakos	Translational & Clinical Research Institute, Newcastle University, UK; Medical School, National and Kapodistrian University of Athens, Greece
	Pierre Bedossa	LiverPat, Paris, France; Translational & Clinical Research Institute, Newcastle University, UK

the dataset [39].

2.5.4. Data monitoring

Monitoring is a crucial quality control process to establish that study activities are being carried out as intended, so that deficiencies can be addressed, and is critical to the protection of human subjects, the conduct of high-quality studies and the generation of robust clinical trial data. [40]. The European NAFLD Registry has adopted a risk-based monitoring approach as recommended by the FDA in its guidance document *Oversight of Clinical Investigations – A Risk-Based Approach to Monitoring (August 2013)* [40,41]. This dynamic, iterative process focuses oversight activities on preventing or mitigating important and likely risks to data quality and trial integrity. Thus, findings determine subsequent actions (for example, targeted additional training of clinical investigators at sites, clarification of protocol requirements) to ensure the requisite levels of data quality are achieved. Due to the non-interventional nature of the Registry, risk-assessment has determined that the likelihood of direct harm to participants is minimal and so monitoring primarily focusses on ensuring that conduct is in compliance with the protocol and that study data are complete, accurate and verifiable. In line with the organisational structure of the Registry, monitoring activities are structured as three tiers: central oversight of data integrity, country-level source-data verification checks with external monitors visiting sites and site-level internal data checks conducted by the local research teams.

Central monitoring is conducted by the central Registry Management Team (Fig. 2). Central data managers perform consistency checks of data entered into the Registry at least every 6 months. These include review of enrolment rates against site and study targets; data validation for completeness and plausibility; and oversight of monitoring activities. Any inconsistencies, errors or omissions detected are flagged to the recruiting site to address. The Registry Management Team and Central Biobank also oversees biological sample management and cross-referencing of the biobank catalogue with the Registry database to ensure samples are received and logged appropriately.

At the country level, National Clinical Leads serve as the national chief investigator in their territory and are responsible for compliance and on-site monitoring. The National Clinical Leads carry out Site Initiation visits providing information to sites on study background, protocol, and data management procedures. Inconsistencies, errors or omissions flagged by the Registry Management Team are highlighted to the National Clinical Lead who ensures the recruiting site checks and amends errors as necessary. A key role of the National Clinical Lead is to appoint site monitors to ensure compliance with GCP, data consistency across sites in a territory and appropriateness of training. Site monitoring includes several important activities, but crucially source data verification (SDV). A first monitoring visit is conducted at each site within three months of the first subject enrolled into the European NAFLD Registry, once per year of the study and until three months after the recruitment of the last subject. Briefly, the first two participants in the study are subject to 100% SDV through the entirety of the project; if major errors/critical findings are found in these patients, the subsequent two patients recruited will follow the same monitoring procedure through the study. Additional activities include review of signed consent forms for all participants and authority approvals are in place.

The final layer of oversight comes at a site-level. Site level monitoring is carried out by an independent member of the local research team, not the individual who entered the data, and includes confirmation of consent, data collection according to study protocol and ensuring complete datasets. Protocol deviations are documented in each participant record using the protocol deviation form and reported to the National Clinical Lead. Internal monitoring tasks include checks that: all participants are consented on the most up-to-date, approved informed consent form in effect at their last visit and that all forms have been completed correctly and the consent date entered into the Registry; data being collected is consistent with what is required by the study protocol and an accurate transcription of the source documents, with special attention being paid to accuracy of reported clinical events and associated investigations.

2.6. Data management

The European NAFLD Registry has a comprehensive data management plan (DMP) to allow secure management of generated data sets and plans for publications, data access and preservation policies. The DMP will be regularly updated to reflect other data sets that may be developed within the European NAFLD Registry, which aims to provide accurate and high-quality data to the research community, so that the Registry will contribute to future advancements in the field of advanced diagnostics. Dissemination of research data is planned in the form of publications in scientific journals and/or presented in scientific or other meetings for the benefit of the wider medical community. Participants will not be identifiable from such published data nor in any datasets that may be placed in the public domain.

3. Discussion

The European NAFLD Registry represents a major international effort involving leading secondary/tertiary care centres in Europe, to prospectively recruit patients into an observational study exploring the pathophysiology of progressive liver disease, disease outcomes and associated predictive factors in patients with NAFLD. Importantly, data generated from this study will address key unmet medical needs: the European NAFLD Registry underpins the LITMUS consortium goal of establishing a defined set of biomarkers that singly or in combination, enable detection and monitoring of disease progression to/regression

from NAFL through NASH to fibrosis and cirrhosis that may be used in clinical trials.

The European NAFLD Registry seeks to cognise the variability in NAFLD disease course by leveraging large sets of integrated '-omic' data to better understand the pathophysiological processes that underpin NAFLD disease severity and progression. It also collates existing prospectively recruited cohorts to permit better granularity of disease outcomes. Together, the European NAFLD Registry data set will allow better correlation of biomarkers with disease diagnosis, prognosis and monitoring, permitting the advent of new pharmacotherapy, which is desperately needed.

The strengths of the Registry are its large sample size, international recruitment, standardised procedures, and fastidious data monitoring plans. The Registry aims not only to collect data of the highest quality, but also to have the ability to prove that the data is of the utmost veracity. Built within the Registry is a geographical hierarchy that facilitates implementation of a clinical monitoring plan that ultimately verifies the high quality of the data collected. A unique strength of the Registry is its ability to provide integrated datasets of both cross sectional and longitudinal analysis *in the same patient* to ascertain causal relationships and mechanisms in disease progression. This capability will facilitate unsupervised data exploration and analysis to develop novel research hypothesis by artificial intelligence/machine learning in addition to end-user directed hypothesis testing. With approved pharmacotherapies for NASH likely to become available in the near future, the European NAFLD Registry platform also has the capability to support authority requested post-marketing surveillance studies (post-authorisation safety and/or efficacy studies); facilitating the systematic monitoring of medications while they are used in clinical practice.

In summary, the European NAFLD Registry is a comprehensive study using standardised data collection practises, clinical monitoring plans and observational protocols to garner the highest quality, large datasets that can be leveraged to better understand and ultimately develop biomarkers across the spectrum of NAFLD severity that can lead to efficient drug discovery and regulatory approval- a key unmet need in NAFLD management. The Registry will facilitate research at scale and pace, with rapid dissemination of research findings to improve patient management and outcomes.

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Authors contributions

Study concept and design: Conceptualiziation, funding acquisition and supervision: QMA; project administration and data curation: KW; writing - original draft: TH, KW, QMA; acquisition of data and critical revision of the manuscript for important intellectual content: all authors.

Declaration of Competing Interest

No authors have conflicts of interest relevant to this paper.

Appendix A. Supplementary data

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