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ABSTRACT

Introduction The prevalence of non-alcoholic fatty liver disease (NAFLD) ranges from 25% in the general population to 90% in patients with obesity scheduled for bariatric surgery. NAFLD can progress towards non-alcoholic steatohepatitis (NASH) associated with complications such as cirrhosis, hepatocellular carcinoma and cardiovascular disease. To date, losing weight and lifestyle modifications are the best known treatments for NASH. Bariatric surgery significantly improves NAFLD/NASH in the short term. However, the extent of this improvement is not yet clear and long-term data on the natural course of NAFLD/NASH after bariatric surgery are lacking. The factors involved in NAFLD/NASH regression after bariatric surgery have not been elucidated.

Methods and analysis This is an observational prospective cohort study including patients scheduled for bariatric surgery. Extensive metabolic and cardiovascular analyses will be carried out including measurements of carotid intima media thickness and pulse wave velocity. Genomic, proteomic, lipidomic and metabolomic studies will be done. Microbioma analyses before and 1 year after surgery will be done. Transient elastography measurements will be performed before and at 1, 3 and 5 years after surgery. For those with an elevated preoperative transient elastography measurement by Fibroscan, a laparoscopic liver biopsy will be performed during surgery. Primary outcome measures are the change of steatosis and liver fibrosis 5 years after surgery. Secondary outcome measure is the comparison of the transient elastography measurements with the NAFLD Activity Score from the biopsies.

Ethics and dissemination The protocol has been approved by the Medical Research Ethics Committees United, Nieuwegein, on 1 March 2022 (registration code R21.103/NL79423.100.21). The study results will be submitted for publication in peer-reviewed journals and data will be presented at scientific meetings.

Trial registration number NCT05499949.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Liver biopsies before surgery, non-invasive tests and multiomics approach before and after surgery.
⇒ Five years follow-up.
⇒ Three types of commonly used procedures (laparoscopic sleeve gastrectomy, Roux-en-Y gastric bypass and one-anastomosis gastric bypass) are studied.
⇒ Single-centre study.
⇒ No histological samples after surgery.

INTRODUCTION

Non-alcoholic steatohepatitis (NASH), usually preceded by non-alcoholic fatty liver disease (NAFLD), is an inflammatory condition with fat accumulation and sometimes development of fibrosis in the liver.[1] Recent studies have shown that NASH is the most frequent cause of liver transplantation in females in the USA and it will soon reach a similar first position in men.[2] At least, 90% of the patients with morbid obesity undergoing bariatric surgery may have NAFLD.[3 4] NAFLD is the most frequent liver disease worldwide, with an estimate of 25% of the population having this disorder.[5–7] At least 20% of patients with NAFLD will progress to NASH.[1] Some ethnicities may also be at higher risk to develop NAFLD and NASH.[6 7] Data in the Netherlands on the prevalence of this condition are scarce.[8 9]

The cause of NAFLD and NASH development remains unclear although it is generally accepted that the presence of insulin resistance is a prerequisite and that a genetic predisposition may play a role in the pathogenesis.[10 11] Gut microbiota composition may also be involved in the pathogenesis of NAFLD/NASH. The intestine barrier permeability may be altered exposing the liver to other bacteria or metabolites that will induce proinflammatory signalling pathways in the liver. In general, alterations in the gut

microbial composition have been frequently observed in individuals with NAFLD and an alteration in plasma metabolites due to microbiota and/or diet has been linked to the development of NAFLD.[12–14]

NAFLD is an independent risk factor for cirrhosis, liver failure, hepatocellular carcinoma, coronary artery disease, chronic kidney disease and colorectal carcinomas.[5 7 15–17] Patients with NAFLD are also more likely to develop type 2 diabetes mellitus[18] and they are at risk for developing cardiovascular disease and heart failure.[19 20]

The global disease burden of NAFLD/NASH is high. European guidelines advocate active screening of NAFLD and NASH in high-risk groups such as patients with diabetes, obesity, hypertension and subjects with the metabolic syndrome.[21 22] For this purpose, a diagnostic flow chart and active screening have been proposed by three different scientific societies, namely the European Association for the Study of Liver, the European Association for the Study of Diabetes and the European Association for the Study of Obesity.

In addition, it is of great importance to determine the severity of the liver fibrosis (according to the Metavir classification) since this largely determines the prognosis.[23] Subjects with stage F3 or F4 of NASH have a significantly worse prognosis than those at stages F2 or lower. Of note, Fibroscan measurements are very sensitive and specific for F1 and F4 fibrosis stages but Fibroscan cannot reliably differentiate between F2 and F3.[24 25] Therefore, confirmation with histology in patients with intermediate to severe fibrosis classified as F2 and F3 by Fibroscan is warranted.

Although a great number of pharmacological interventions are being investigated, so far none has been approved for clinical use.[26 27] The cornerstone of the treatment at present is to lose weight by lifestyle management and improved nutritional interventions. However, the evidence for this approach is based on small studies including a limited number of subjects and with limited follow-up.[28] Different lifestyle interventions have been described with the Mediterranean diet being the most efficient.[29] Bariatric surgery is the most effective surgical intervention to decrease body weight[30–34] and it may also lead to NAFLD/NASH regression. Bariatric surgery is usually part of an extensive protocol with a long-term follow-up where attention is given to lifestyle changes. Data on the possible remission of NASH after bariatric surgery are limited.[1] In one systematic review, 9940 subjects were included in 45 studies of which 2 studies reported follow-up with liver biopsies. Only seven studies were comparative, evaluating Roux-Y-Gastric Bypass (RYGB) with sleeve gastrectomy. All other studies only evaluated biochemical variables. So far, no study has included measurements of liver fibrosis with transient elastography in the follow-up.[35]

In this study, we aim to investigate the prevalence and the severity of NAFLD/NASH in patients enrolled for bariatric surgery in the Netherlands, the association with other comorbidities and the effect of bariatric surgery on the resolution of these disorders. We also aim to establish a correlation between histology (liver biopsy) and transient elastography measurements in these patients. By publishing this protocol, we aim to create awareness of this condition and to encourage research within this topic.

METHODS AND ANALYSIS

Study design and participants

This is an observational prospective cohort study. It will take place in a single obesity centre in the Netherlands in a large teaching semiacademic municipal hospital: the Franciscus Gasthuis & Vlietland Hospital. We aim to include 300 patients from the regular morbid obesity bariatric programme from 1 September 2022, with a 5-year postoperative follow-up. Participants must fulfil the criteria of the International Federation for the Surgery of Obesity at screening. In order to participate in this study, subjects have to speak and read Dutch in order to provide written informed consent. Patients who meet any of the following criteria at screening will be excluded from the study: participants younger than 18 years or older than 65 years, participants with an established diagnosis of another primary liver disease, histologically documented liver cirrhosis (fibrosis stage F4) at screening or in a historical biopsy, participants with active HIV infection and/or treatment and participants with diagnosed malignancies with or without active treatment. Moreover, participants with a history or evidence of any other clinically significant condition or planned or expected procedure that may compromise the patient's safety or ability to complete the study will be excluded.

The measurements will be performed at the specified time points, at the Department of Endocrinology, Vascular Medicine and Diabetes of our hospital (table 1).

After the 5 years follow-up, patients will be referred back to the general practitioner following international guidelines and our local protocol. After bariatric surgery, patients will be referred to the department of Gastroenterology and Hepatology for further follow-up in case of persistent abnormal elastography measurements (F2 with elevated liver enzymes or ≥F3) (see figure 1).

The type of surgery will be determined by the surgeon together with the patient. The type of operation offered, depends on several clinical characteristics (comorbidities, medication use, etc) and this decision will not be influenced by participation in this study. In our hospital, we use three types of surgery: gastric sleeve resection, RYGB and one-anastomosis gastric bypass.

Patient an public involvement

Patients were not involved in the design and conduct of this research. During the feasibility stage, priority of the research question, choice of outcome measures and methods of recruitment were informed by discussions with the medical team. During the trial, no patient will join the research team. Once the study is published, participants will be informed of the results on their demand.

Table 1 Timetable and intervention planning

Time	Subject n=	Enrolment	Visit 1	Surgery	FU: moment after surgery		
		−2 months	0 month	3 months	15 months (1-year FU)	39 months (3-year FU)	63 months (5-year FU)
Weighing	300	x	x	x	x	x	x
Genetic analysis	300		x				
Metabolomics analysis	300			x	x		
Transient elastography	300		x	x	x	x	x
Lifestyle coaching	300		x				
IMT measurement	300		x		x	x	x
PWV measurement	300		x		x	x	x
Faeces analysis	300		x		x		
Heart echography	60		x		x		
Liver biopsy (if ≥F2)	60			x			

Timetable and intervention planning.
FU, follow-up; IMT, intima media thickness; PWV, pulse wave velocity.

Outcome measures

The primary outcome measure is to evaluate NAFLD/NASH changes in terms of steatosis and liver elasticity in patients with morbid obesity at 5 years after bariatric surgery. Therefore we will use the Fibrosis-4 (FIB4) score[36] and then proceed with evaluation using transient elastography (Fibroscan, Paris, France) before surgery and at 1, 3 and 5 years after surgery. The secondary outcome measure is to establish the correlation between histology (liver biopsy) and transient elastography measurement in a population with morbid obesity. For this purpose, a laparoscopic liver biopsy will be performed during the bariatric procedure in patients with an elevated measurement with transient elastography (≥7.95 kPa, see table 2)

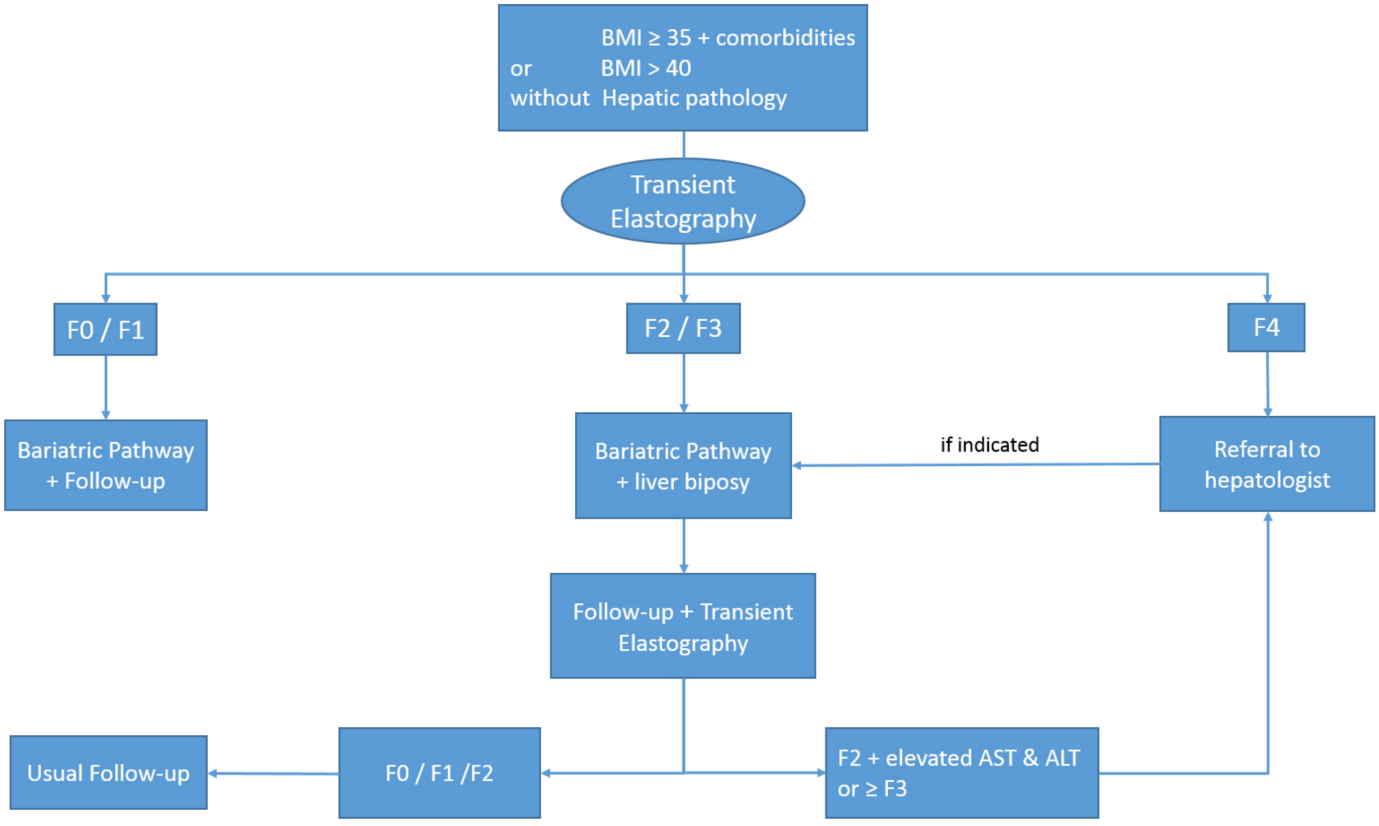

Figure 1 Decision tree for liver biopsy and referral to hepatologist. The Fibrosis score (F) corresponds to the data obtained with transient elastography using transient elastography as given in table 2. ALT, Alanine Transaminase; AST, Aspartate Transaminase; BMI, body mass index.

Table 2 Cut-off values for the evaluation of liver firosis with Fibroscan according to Theel et al[44]

Fibrosis stage	F0	F1	F2	F3	F4
E (kPa)	<6.9	6.9–7.94	7.95–8.94	8.95–14.24	≥14.25

Cut-off values for the evaluation of liver fibrosis with Fibroscan according to Theel *et al*.[44]
E, elasticity.

estimated preoperatively. Two pathologists will score independently the liver biopsies (NAFLD/NASH status using the NAFLD Activity Score (NAS)).[37] In case of disagreement on the classification, a third pathologist will be consulted and the score set by 2 out of 3 pathologists will be recorded.

In case of perioperative complications, all (serious) adverse events will be registered to evaluate the safety of the surgery procedure for the participants. During follow-up, we will also record all cases of liver failure, decompensation, hepatocarcinoma or need for transplantation in order to gain insight in the pathophysiology of NAFLD and weight loss as well as other cardiovascular and renal complications and death. Besides, participants needing a (laparoscopic) cholecystectomy after the bariatric procedure will be registered.

The Franciscus Obesity NASH Study also aims to evaluate determinants of liver steatosis and elasticity. Therefore, subanalyses will be carried out in relation to the type of bariatric surgery (gastric sleeve resection, RYGB, one-anastomosis gastric bypass), the presence of diabetes and if indicated the use and type of medication (insulin, oral medication, GLP1 analogues), the polymorphism of genetic markers (among others: PNPLA3, TM6SF2, MBOAT7, GCKR and HSD17B13), the effect of lifestyle intervention and the Firmicutes/Bacteroides ratio in the microbioma.

Metabolomics, lipidomics and proteomics analyses will be performed in order to identify potential biomarkers of NASH.

Full panels of metabolites from EDTA-plasma samples will be 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 small molecules including amino acids, central energy and carbon metabolism regulators, together with exogenous molecules such as dietary chemicals, microbiome-derived metabolites, environmental chemicals, commercial products and drugs. A lipidomics platform will allow the analysis and classification of more than 27 lipid classes including diglycerides, triglycerides, phospholipids, ceramides, sphingolipids and many more. A targeted MS/MS platform will be used to cover more than 300 modified free fatty acids that act as bioactive lipid mediators, both locally and systemically, and that are involved in innate immunity, inflammation, chemotaxis, cell survival, cell proliferation, differentiation and apoptosis. Targeted and untargeted proteomics (SomaScan, O-link panels) will be used to identify serological protein biomarkers, which will then be validated through ELISAs or Multiplex assays.

A cardiovascular analysis will also be performed including measurements of carotid intima media thickness, pulse wave velocity and echocardiography to determine if changes in liver fibrosis stage are associated with change in cardiovascular function. Moreover, any cardiovascular event will be registered in order to evaluate the risk of cardiovascular complications in participants with different stadia of NAFLD/NASH.

Data management

Patient data and material will be coded and handled confidentially. For each subject, a unique identification code (neither based on patient initials nor birth date) will be used to link the study data to the subject's personal characteristics. The key to this code is safeguarded by the principal investigators, who also have access to the source data. Data will be stored using Castor EDC (Amsterdam, the Netherlands), a web-based electronic data capture software program for clinical research. The database will be accessible by the study team only and according to study team role if applicable.

Sample size

Based on a recent study,[38] we expect to find a mean elastography of 9.7 kPa (SD 4.4) before surgery and a mean elastography of 7.2 kPa (SD 4.0) 5 years after bariatric surgery. Using a two-sided t-test of equal means with 5% significance and at least 80% power, at least 25 subjects (paired observations) will be required for the analysis of bariatric surgery on NASH changes based on elastography.

We expect to have a considerable drop out during follow-up. The study of van de Laar *et al* provided information on follow-up rate for Dutch patients 5 years after bariatric surgery (783 patients after 5 years from 9393 before surgery or 8.33% from the total).[39] Thus, given this low follow-up rate and the fact that calculations indicate that we need at least 25 patients with 5 years follow-up to answer our primary objective, we will need to include at least 300 patients (300×8.33%=25) before surgery.

According to the literature[6 40 41] about 20% of our subjects scheduled for bariatric surgery are expected to demonstrate histological abnormalities reflecting NASH (≥F2). Therefore, from the 300 subjects enrolled in our study, about 60 of these may be eligible for a laparoscopic liver biopsy during the bariatric procedure to determine their actual degree of fibrosis.

Statistical analysis

This study is designed as an observational, prospective, non-randomised study. For statistical analysis, IBM SPSS V.28.0 (IBM) will be used. Efforts will be made to prevent missing data by checking the completeness of the collected data. Missing data may be expected due to the low follow-up rate of this patient population or failure of transient elastography measurements. Difference in steatosis and liver elasticity will be analysed comparing the measurements before and 5 years after bariatric surgery using a paired t-tests (if normally distributed otherwise the Wilcoxon signed rank test). We aim to compare the classification of the liver fibrosis (Metavir F1-4) according to the TE measurement with the NAS score from the biopsies. For this, we will use a χ^2 test for trend to determine if there is any significant difference of classification between the two methods. P values <0.05 (two tailed) will be considered statistically significant.

DISCUSSION

In the last decades, the prevalence of obesity has risen rapidly in most parts of the world, closely associated with comorbidities such as NAFLD and its more severe form NASH. Multiple studies estimate that NAFLD may be present in 17%–33% of the world population[5 42] but little is known about the prevalence in patients enrolled for bariatric surgery and the evolution after such a procedure. In this study, a consistent analysis of these patients will give insight into the preoperative prevalence of patients with NAFLD, those who are more at risk for NASH and the metabolic changes induced by bariatric surgery. A weakness of our study is that we will not perform a liver biopsy in all participants but only in those with a preoperative elevated liver elasticity measured with transient elastography.

Bariatric surgery has proven its long-term effect on weight loss and it currently remains the best therapeutic option to maintain weight loss.[32] The causal relationship with obesity is reflected by the fact that after weight loss, NAFLD decreases significantly, although not in every patient and not to the same extent.[30 43] This study aims to determine through different measurements which bariatric procedure and which mechanism improves liver steatosis and fibrosis at 5 years. The high drop-out rate may constitute an important limitation. This point will be analysed and discussed. Special attention will be given in the correction of possible disparities in age and gender.

NAFLD is often underdiagnosed in patients with obesity. The reason for that is often a lack of knowledge and equipment to evaluate the risk of developing serious events. To our knowledge, the literature does not provide specific cut-off values for the bariatric population to distinguish patients with a higher risk.[44] The study will use transient elastography measurements and will compare these with histological findings in order to establish cut-off values.

This study will expand the current knowledge on NAFLD/NASH in severe obesity and we will be able to investigate in detail changes in pathophysiological and cardiovascular mechanisms associated with severe obesity in these subjects. The aim is to identify markers and metabolic pathways that will help to identify subjects at high risk of developing complications due to NAFLD.

ETHICS AND DISSEMINATION

The study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The study protocol was approved by the IRB and the regional MEC-U, Utrecht, the Netherlands (protocol number R21-103). The study results will be submitted for publication in a peer-reviewed journal and at conference presentations.

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Contributors WT was the main author of the manuscript. BMB-dK revised the statistical part. FD-H and RDK revised the hepatological part. EvR, DAK, BMvD and JAA advised on the feasibility of the protocol and critically reviewed the manuscript. BN, EvdZ, TH, DEG and JW have contributed to the study design and helped to solve logistical problems. MCC supervised the project and substantially contributed to the conception and design of the work.

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Competing interests None declared.

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