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master in de biomedische wetenschappen

Masterthesis

Non-Alcoholic Fatty Liver Disease and Diabetes Mellitus Type 2: Unveiling specific characteristics and risk factors

Ece Tosun

Scriptie ingediend tot het behalen van de graad van master in de biomedische wetenschappen, afstudeerrichting klinische biomedische wetenschappen

PROMOTOR :

Prof. dr. Geert ROBAEYS

BEGELEIDER :

Mevrouw Leen HEYENS

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Non-Alcoholic Fatty Liver Disease and Diabetes Mellitus type 2: Unveiling specific characteristics and risk factors

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*Body Mass Index as a risk factor of NAFLD in T2DM

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ABSTRACT

BACKGROUND: Non-alcoholic fatty liver disease (NAFLD) and Diabetes Mellitus type 2 (T2DM) are the most common metabolic Western lifestyle-related diseases, characterized by the metabolic syndrome (MetS). The growing T2DM epidemic causes an alarming increase in NAFLD prevalence. Despite its high prevalence, NAFLD remains under-recognized. Only limited information about specific risk factors is known in T2DM patients. Therefore, this study aims to profile T2DM patients with NAFLD.

METHODS: A cross-sectional study was carried out with 259 T2DM patients. To evaluate liver stiffness and steatosis, all patients underwent a standard-of-care FibroScan® measurement, and noninvasive test scores were calculated. Clinical parameters (BMI, waist circumference) were measured, and medication usage was monitored. From the patient's records, laboratory data and medical backgrounds were collected. The associations between liver stiffness, steatosis and characteristics, and risk factors were assessed using multiple regression models.

RESULTS: A significant positive association was found between vibration-controlled transient elastography values and gamma-glutamyl transferase, controlled-attenuation parameter (CAP) values and triglycerides and fatty liver index values and the presence of MetS. A significant negative correlation was found between CAP values and insulin therapy. In all models, liver stiffness and steatosis were significantly associated with the BMI.

CONCLUSION: Our results demonstrate, in contrast with literature, that specific blood parameters are linked to NAFLD. Moreover, insulin therapy has a protective effect on steatosis. The presence of MetS and an increased BMI remain substantial risk factors for NAFLD. These findings form an essential basis to elucidate the complex relationship between T2DM and NAFLD.

INTRODUCTION

Non-alcoholic Fatty Liver Disease (NAFLD) is known to be one of the most common chronic liver diseases in the Western world. Nowadays, it is a significant cause of non-liver and liver-related mortality and morbidity (1, 2, 3). This chronic liver disease is characterized by 5% or more hepatic fat accumulation without the overconsumption of alcohol or other causes of liver steatosis (2). The prevalence of NAFLD was estimated to be between 25 and 32.4% worldwide and in Belgium, continuing to increase significantly compared to previous years (1). This indicates that in a population of 11.5 million, 2.87 million suffer from NAFLD (4). Alarmingly, in our Western world, NAFLD is predicted to become a crucial indication for liver transplantation by 2030 (5, 6). One of the most important factors in the early development of NAFLD is weight gain. An unhealthy lifestyle is characterized by a high-calorie diet and decreased physical activity. The liver plays a crucial role in all metabolic processes in the human body, including maintaining metabolic balance. However, overnutrition, causing insulin resistance (IR) and obesity, generates multiple insults to metabolic liver features such as hepatic lipid accumulation. This leads to metabolic dysregulation in the liver, the driving force of NAFLD development (4, 7, 8). Additionally, the presence of other comorbidities, such as Diabetes Mellitus type 2

(T2DM), is also associated with hepatic fat accumulation due to IR. This makes T2DM a major risk factor for developing NAFLD (9).

NAFLD spectrum

NAFLD is a multifactorial and heterogeneous disease since it is associated with other comorbidities such as T2DM, an unhealthy lifestyle, and metabolic dysregulation. Moreover, this heterogeneous disease is characterized by different disease entities and its progression from one entity to another (4, 10) **(Figure 1).** Disease progression can start from a healthy liver to a non-alcoholic fatty liver (NAFL) (25-30%), representing simple steatosis without or with little inflammatory changes. NAFL can evolve into non-alcoholic steatohepatitis (NASH), considered as a progressive form of NAFLD. NASH is histologically characterized by chronic inflammation, cell damage, lobular inflammation, and ballooning of hepatocytes. Eventually, NASH can evolve into liver fibrosis which in turn is the driving force of irreversible end-stage liver entities, cirrhosis, and decompensated cirrhosis (4, 11). Moreover, the progression of the disease may even result in the development of hepatocellular carcinoma (HCC) (12). The liver disease in most patients can be stable and slowly progressive, not resulting in an advanced disease stage such as cirrhosis or HCC. However, a small proportion of patients reach end-stage liver diseases. Moreover, many patients are asymptomatic until they reach end-stage liver diseases (13). Namely, 20-25 % of NAFLD patients will develop NASH over the course of three to seven years, nearly 9-25 % of NASH patients will develop cirrhosis in 10 to 20 years, and about 8 % of cirrhosis patients will have HCC in five years (4, 11, 13, 14). Alarmingly, 2% of NAFLD patients have the possibility to develop HCC in 8 years (11).

As mentioned earlier, many NAFLD patients are asymptomatic or show non-specific symptoms. The primary abnormality in NAFLD is mainly characterized by mildly elevated liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT). However, in most of the patients, the liver enzymes appear to be normal (15). Furthermore, the most common symptoms are fatigue, upper abdominal pain, bloating, and sleep disturbances. Patients suffering from end-stage liver diseases such as cirrhosis or HCC will present more severe specific symptoms such as ascites, portal hypertension, hepatomegaly, and splenomegaly (15, 16). NAFLD is also known to cause non-liver-related mortality. Hence, growing evidence indicates that patients with NAFLD have an increased risk of cardiovascular morbidity and mortality. Moreover, the combination of T2DM and NAFLD accelerates the development of cardiovascular diseases (17, 18).

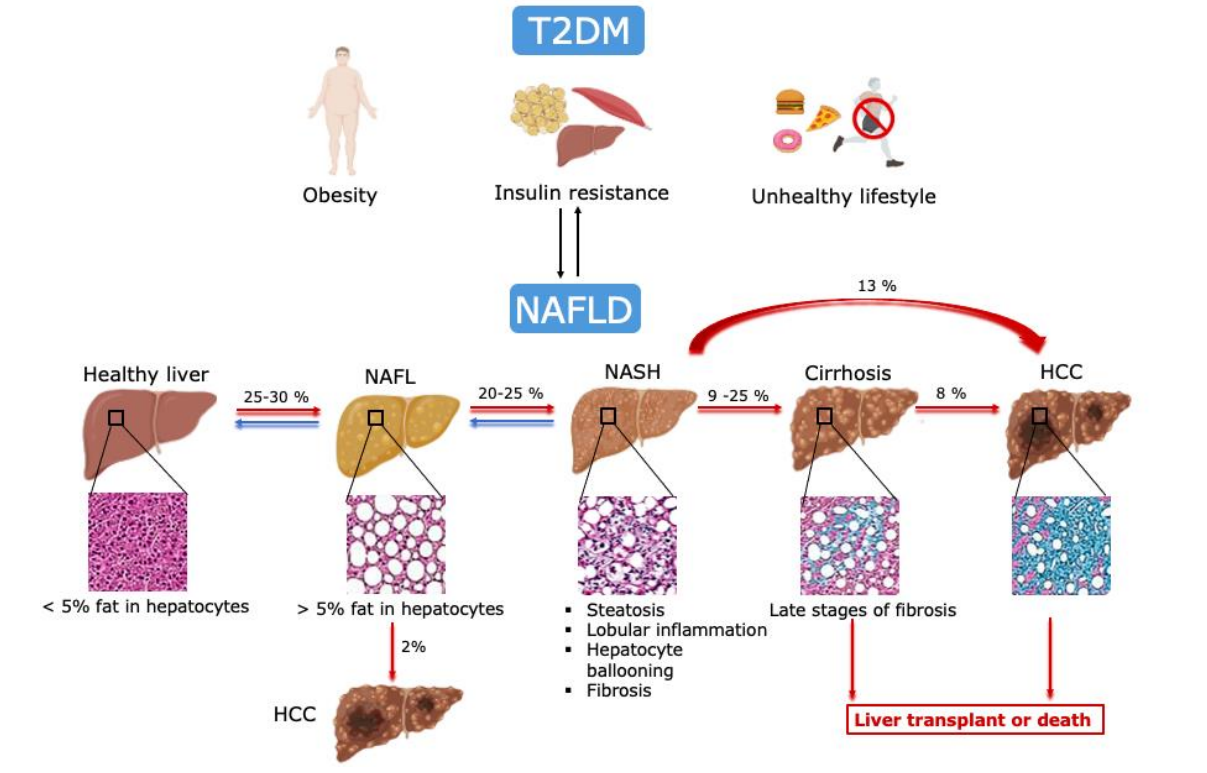

Figure 1.Non-alcoholic fatty liver disease and Diabetes Mellitus type 2. T2DM; Diabetes Mellitus type 2, NAFLD; non-alcoholic fatty liver disease, NAFL; non-alcoholic fatty liver, NASH; non-alcoholic steatohepatitis, HCC, hepatocellular carcinoma.

NAFLD diagnosis

Since the prevalence of NAFLD keeps rising and because of its progressive development, screening specific risk populations such as T2DM has been crucial. Moreover, accurate and early diagnosis is beneficial for the patients to be one step ahead from further progression (19). Diagnosis of NAFLD is based on combining different modalities, each with its advantages and disadvantages. Nowadays, liver biopsy is still considered the gold standard for NAFLD diagnosis, especially for differentiating NASH from simple steatosis (NAFL) (15, 17, 20). However, this method is limited in early NAFLD screening due to its associated costs and invasive nature. The size of a liver biopsy only represents 1/50.000 of the entire liver tissue hence leading to a possible incorrect diagnosis. Consequently, all guidelines agree that biopsies should only be performed on select individuals. To address these limitations, numerous noninvasive methods have been developed for diagnosing steatosis and fibrosis (20, 21). Ultrasound elastography has been widely used primarily to detect hepatic steatosis and fibrosis. The first device developed to measure liver elasticity as an alternative for liver biopsy is the FibroScan® *(Echosens, Paris, France)*. Both steatosis and liver stiffness can be measured based on two parameters provided by the FibroScan®: vibration controlled transient elastography (VCTE) and controlled attenuation parameter (CAP) (19, 22, 23). In the last decades, various noninvasive test scores have also been developed based on blood parameters to indicate the rate of steatosis and fibrosis in patients (24, 25). The Fibrosis Index Based on 4 Factors (FIB-4) score and NAFLD fibrosis score (NFS) are widely used to evaluate the fibrosis grade since they only require standard laboratory data and clinical parameters. Based on the specific result, both scores can either rule in our rule out advanced fibrosis (21, 24). Furthermore, according to the European Association for the Study of the Liver (EASL), the fatty liver index (FLI) can identify steatosis. Recently, the FibroScan-AST (FAST™) score has been developed to identify patients with progressive NASH (26, 27). In the first-line examination, ultrasonography (US) is mainly preferred to detect steatosis because of its low cost and availability. It is generally a primary practice to screen for NAFLD. Furthermore, Computed Tomography (CT) is more accurate than US in grading moderate to severe steatosis, but limited in its capability to detect mild steatosis. Besides, its radiation exposure limits its use as a screening tool (21). Lastly, magnetic resonance imaging (MRI) accurately detects hepatic steatosis. However, its use is limited in conventional MRI due to T1 bias and T2 decay and the signal interference caused by protons in fat (28).

NAFLD risk factors

The significantly growing epidemic of T2DM and obesity gives rise to the alarmingly increased prevalence of NAFLD (29, 30). These diseases are highly associated with environmental factors such as dietary patterns (e.g., excessive food consumption or processed food) and a sedentary lifestyle, reinforcing NAFLD development (31, 32, 33). The degree of steatosis was found to be correlated with the body mass index (BMI), waist circumference, and metabolic syndrome (MetS), which are significant characteristics of T2DM (34). Furthermore, according to the World Health Organization (WHO), MetS is characterized by abdominal obesity, T2DM, hyperlipidemia, hypercholesterolemia and, arterial hypertension (AHT). These features also impact liver health and are associated with NAFLD (35). Moreover, numerous studies have described metabolic risk factors for developing NAFLD, especially dyslipidemia and insulin resistance (9, 13, 36). Consequently, hyperglycemia and hyperlipaemia lead to a dysregulated lipid metabolism in the liver leading to hepatic fat accumulation (15).

The growing outbreak of T2DM has been leading to an increased prevalence of NAFLD worldwide. In 2021, the International Diabetes Federation (IDF) estimated that 536.6 million people had Diabetes globally (37). Moreover, this number is predicted to keep rising significantly. Large meta-analyses of observational studies have estimated that the global prevalence of NAFLD in patients with T2DM is approximately 40-70 %. Importantly, higher rates were found in Europe (68%) (38). These significant numbers and findings emphasize the significant association between both diseases and that in most of the patients, NAFLD and T2DM coexist (13, 39, 40). Prospective studies have shown that T2DM is an independent risk factor for NAFLD development, progression, and liver-related mortality (41). Moreover, T2DM is associated with a more than two times higher risk of advanced fibrosis and cirrhosis-related complications compared to patients without T2DM (13). This increased risk is alarming since fibrosis and cirrhosis stages are significantly associated with increased mortality and liver-related morbidity (38). Furthermore, poor glycemic control, particularly hyperglycemia and hyperinsulinemia, have been described as significant predictors in the development of hepatic fibrosis (40). Even though a significant association between both prevalent diseases has been established, limited knowledge is available regarding the specific characterization of NAFLD in the presence of T2DM. This gives rise to the inability to develop clear approaches for the care and management of these patients (42).

Aim of the study

Despite the significant rise of the disease globally, NAFLD remains highly under-recognized among healthcare and the wider community and is relatively complex for healthcare physicians. Furthermore, no standardized care path or specific treatment is available globally. Recognition of the diversity in disease progression as well as the factors that impact disease progression, is crucial to create guidance for patient care, especially in specific risk populations such as T2DM (13, 14, 39). Preliminary research describes specific characteristics and risk factors such as BMI, IR, disrupted liver enzymes, and elevated lipid profile that are crucial in developing NAFLD (40). However, little information is available regarding the specific risk factors and their association with different NAFLD disease stages in T2DM patients. Therefore, in this observational interventional cross-sectional study, we aim to profile T2DM patients suffering from NAFLD.

MATERIALS AND METHODS

Study design

The NaDia study (NCT04999124) is a monocentric observational cross-sectional study in Ziekenhuis Oost-Limburg (ZOL) (Genk, Belgium), to profile T2DM patients with NAFLD.

Ethical approval

The ethical committee of ZOL and Hasselt University approved the study protocol in 2019. The study was carried out according to the Declaration of Helsinki. All study participants provided written consent after being informed of the purpose and implications of the study.

Study population

The study eligibility of the patients was based on predefined inclusion and exclusion criteria **(Table 1).** T2DM patients were referred by their endocrinologist to perform the standard-of-care (SoC) FibroScan® *(Echosens, Paris, France)* measurement. A total of 368 patients were included in the study.

Definitions

T2DM diagnosis of the patients was based on their diagnosis present in the electronic patient records (EPD). The reference value of regulated glycemia is an HbA1c value < 6 %, according to the laboratory of ZOL. T2DM patients belong to a specific care trajectory based on their therapy needs. "Diabetes Conventie" trajectory patients are treated with at least two insulin injections and additional oral antidiabetics. "Zorg" trajectory consists of patients treated with one insulin injection and additional oral antidiabetics. "Prediabetes" trajectory patients are only treated with oral antidiabetics and are not followed up at the hospital. The following criteria define MetS according to the IDF: A high waist circumference must be present (≥ 90 cm in males and ≥ 80 cm in females) with the addition of two of the following criteria to diagnose a patient with MetS: arterial hypertension (AHT) (≥ 130/85 mmHg or treatment for AHT or previously diagnosed with AHT), hyperglycemia (fasting glucose ≥100 mg/dL or previously diagnosed with T2DM), high triglycerides (TG) (TG ≥150 mg/dL or specific treatment for this lipid abnormality) and reduced high-density lipoprotein (HDL) cholesterol (<40 mg/dL in males and <50 mg/dL in females or specific treatment for this lipid abnormality) (43). NAFLD diagnosis is based on a CAP value of > 215 dB/m (23).

Clinical measurements

During the one-time study visit, specific body measurements were collected, including height (cm), weight (kg), and waist circumference (cm). The BMI was defined (kg/m^2) as normal (18.50 – 24.99), overweight (≥ 25.00), and obese (≥ 30.00). The waist circumference was

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Diagnosis of T2DM Able to give informed consent in Dutch Able to understand the information sheet and comply with the study protocol Age ≥ 18 years	Excessive alcohol use: - > 20g/ day or 2 units for females - > 30g/ day or 3 units for males Other liver diseases: - Viral causes: Hepatitis B, Hepatitis C - Systemic disorders: Hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency - Auto immune disorders: autoimmune hepatitis, primary biliary cholangitis Secondary causes for steatosis: - Hepatitis C Genotype 3 - Celiac disease - Use of medications: amiodarone, tamoxifen, methotrexate, corticosteroids

T2DM, Diabetes Mellitus type 2.

measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest (44). The smoking state, alcohol consumption, and the patient's current medication intake were actively asked and gathered.

FibroScan ® measurement

In this study, the FibroScan® Mini 430 *(Echosens, Paris, France),* equipped with medium (M) and extra-large (XL) probes, was used. The probe choice is based on a probe selection algorithm of the device displayed in real-time. This algorithm is based on the probe to liver capsule distance. All T2DM patients underwent a FibroScan® measurement to assess their liver fibrosis and steatosis by trained study conductors. Liver stiffness was graded based on the VCTE parameter, which measures the velocity of a low-frequency elastic shear weave propagating through the liver. The results were provided in kilopascals (kPa). The CAP parameter was used to classify the liver steatosis. This parameter estimates the degree of steatosis based on the attenuation of the ultrasound signal of the FibroScan® device. The results were expressed in decibels/meters (dB/m). A FibroScan® measurement was considered valid based on the following criteria: sober state of the patient for at least four hours, at least ten valid measurements and an interquartile range less than 30 % of the median liver stiffness measurement (LSM) value defined in VCTE. To divide the specific liver stiffness and steatosis degree of the patients, the cut-off values described in the Belgian Association for Study of the Liver Guidance document (BASL) and Baveno VII guidelines were used (23, 45).

The liver stiffness can be divided into little to no fibrosis (F0-1), moderate fibrosis (F2-3), and severe fibrosis (F4) **(Table 2).** Liver fat buildup or steatosis can either be presented in no steatosis (S0), little steatosis (S1), moderate steatosis (S2) and severe steatosis (S3) **(Table 3).**

Noninvasive test scores

The following noninvasive test scores were calculated: FIB-4, NFS, FLI, and FAST scores **(Table 4).** Both the FIB-4 score and NFS can rule in or rule out advanced fibrosis. The FAST score was calculated to either rule in or rule out NASH in patients with moderate to advanced fibrosis (stage 2 or higher). To calculate this score, the AST value must be present within six months of the LSM and CAP measurement of the FibroScan®.

Data collection

All the prospective data was written on a paper case report form (CRF) during the one-time study meeting. The data collected on the paper CRF were then filled in the electronic data capture (EDC) system *Castor*. Retrospectively, the following data were collected from the EPD of ZOL (HiX): medical history, comorbidities, and laboratory data**.**

Table 2. Cut-off values to evaluate liver stiffness levels (23, 45).

Type of probe	Cut-off values VCTE (kPa)	Fibrosis grade
M-probe	<7.9	F0-1
	7.9-10.3	F2-3
	>10.3	F4
XL-probe	<7.2	F0-1
	7.2-7.9	F2-3
	>7.9	F4
Baveno VII	5.0-7.0	F0-1
	10.0-15.0	F2-3
	>15.0	F4

VCTE, vibration controlled transient elastography; M, medium; XL, extra-large; F, fibrosis; kP, kilopascals.

Table 3.Cut-off values to evaluate steatosis levels (23, 45).

Cut-off values CAP (dB/m)	Steatosis grade
< 215	S0
215-252	S1
252-296	S2
>296	S3

CAP, controlled attenuation parameter; S, steatosis; dB/m, decibels per millimeter.

Table 4.Noninvasive test scores to evaluate liver stiffness and steatosis levels.

Test acronym	Formula	Cut-off
FIB-4	Age (years) x AST (IU/L)/platelet count x ALT (IU/L)	<65 years: Low risk AF: <1.30 Gray zone: 1.30-2.67 High risk AF: >2.67 >65 years: Low risk AF: <2.00 Gray zone: 2.00-2.67 High risk AF: >2.76
NFS	-1.675+0.037 x age (year) + 0.094 x BMI (kg/m^2) + 1.13 x IFG/ DM (with=1, without=0) + 0.99 x AST/ALT ratio -0.013 x platelets (x10^9/L) – 0.66 x Alb (g/dL)	Absence of AF: <-1.455 Gray zone: -1.455 - 0.676 Presence of AF: >0.676
FLI	(e^0.953*loge(triglycerides) + 0.139*BMI + 0.718*loge(GGT) + 0.053*waist circumference – 15.745)/1+e^0.953*loge(triglycerides) + 0.139*BMI + 0.718*loge(GGT) + 0.053*waist circumference – 15.745)	Rule out steatosis:< 30.0 Gray zone: 30-60 Rule in steatosis: ≥ 60.0
FAST	{(e^1.65 + 1.07 x ln (LSM) + 2.66 x 10^{-8} x CAP^3 -63.3 x AST^{-1})}/{1+ e^1.65+1.07 x ln (LSM) + 2.66 x 10^{-8} x CAP^3-63.3 x AST^{-1})}	Rule out NASH: <0.35 Gray zone: 0.35-0.67 Rule in NASH: 0.67

FIB-4, Fibrosis Index Based on 4 Factors; NFS, NAFLD Fibrosis score; FLI, fatty liver index; FAST score, FibroScan-AST; AST, aspartate aminotransferase; ALT, alanine aminotransferase; IUL, international units per liter; BMI, body mass index; kg, kilograms; m, meters; IFG, impaired fasting glucose; DM, Diabetes Mellitus; Alb, albumin; g/dl; grams per deciliters; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; AF, advanced fibrosis.

Statistical analysis

Statistical analysis of all the gathered data was accomplished with *IBM SPSS v28*. Descriptive statistics describing the specific characteristics of the study population were carried out with frequencies and median (IQR1 -IQR3), and mean ± SD depending on the normal distribution of the data. Multiple linear regression models were built to study the association between NAFLD and explicit characteristics and risk factors. NAFLD was defined as the LSM and CAP, and the noninvasive test scores FIB-4, NFS, and FLI. Explicit characteristics and risk factors were gathered based on a thorough literature search (13, 15, 34, 39, 42). The main characteristics that were tested consist of liver enzymes, parameters of the fat metabolism and the iron metabolism. The main risk factors chosen were age, BMI waist circumference and MetS.

Additionally, the T2DM care trajectory, T2DM-specific medication and dyslipidemia medication were added in the analysis to assess their protective effects. The following assumptions were tested and met after logarithmic transformation of the data: independence of the residuals, homoscedasticity, no collinearity between independent variables and normality of the residuals. The beta coefficients were considered significant with p-values < 0.05, a 95 % confidence interval. The characteristic models contain blood parameters such as liver enzymes and components of the fat metabolism **(Table 5).** The risk factor models contain age, gender BMI, anti-diabetics (insulin, Dipeptidyl-4 inhibitors (DPP-4), Sodium glucose cotransporter-2 (SGLT-2) inhibitors, and glucagon-like-1 peptide (GLP-1) analogues), lipid medication, MetS and HbA1c **(Table 6, Table 7, Table 8).**

Table 5. Characteristic models for liver stiffness and liver steatosis: Blood parameters.

Model 1	Model 2	Model 3
VCTE ALT, GGT, cholesterol, HDL, LDL, TG	**FIB-4** GGT, cholesterol, HDL, LDL, TG	**NFS** GGT, cholesterol, HDL, LDL, TG
Model 4	**Model 5**	**Model 6**
CAP ALT, GGT, TG	**CAP** HDL, ferritin	**FLI** HDL, ferritin

VCTE, vibration controlled transient elastography; FIB-4, Fibrosis Index Based on 4 Factors; NFS, NAFLD fibrosis score; CAP, controlled attenuation parameter; FLI, fatty liver index; ALT, alanine transaminase; GGT, gamma glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

Table 6.Risk factor models for liver stiffness and steatosis: Medication use.

Model 1	Model 2	Model 3	Model 4	Model 5
VCTE Age, BMI, Gender, Insulin, DPP-4, SGLT-2, GLP-1, statin	**FIB-4** BMI, Gender, Insulin, DPP-4, SGLT-2, GLP-1, statin	**NFS** Gender, Insulin, DPP-4, SGLT-2, GLP-1, statin	**CAP** BMI, Gender, Insulin, DPP-4, SGLT-2, GLP-1, statin	**FLI** Age, Gender, Insulin, DPP-4, SGLT-2, GLP-1, statin

VCTE, vibration controlled transient elastography; FIB-4, Fibrosis Index Based on 4 Factors; NFS, NAFLD fibrosis score; CAP, controlled attenuation parameter; FLI, fatty liver index; BMI, body mass index; DPP-4, dipeptidyl peptidase-4; SGLT2, sodium glucose cotransporter; GLP-1, glucagon-like peptide 1.

Table 7. Risk factor models for liver stiffness and steatosis: HbA1c and Metabolic Syndrome.

Model 1	Model 2	Model 3	Model 4	Model 5
VCTE Age, BMI, Gender, HbA1c, MetS	**FIB-4** BMI, Gender, HbA1c, MetS	**NFS** Gender, HbA1c, MetS	**CAP** Age, BMI, Gender, HbA1c, MetS	**FLI** Age, Gender, HbA1c, MetS

VCTE, vibration controlled transient elastography; FIB-4, Fibrosis Index Based on 4 Factors; NFS, NAFLD fibrosis score; CAP, controlled attenuation parameter; FLI, fatty liver index; BMI, body mass index; HbA1c, Hemoglobin A1c; MetS, metabolic syndrome.

Table 8. Risk factor models for liver stiffness and steatosis: Care trajectories.

Model 1	Model 2	Model 3	Model 4	Model 5
VCTE Age, BMI, Gender, DC, ZT	**FIB-4** BMI, Gender, DC, ZT	**NFS** Gender, DC, ZT	**CAP** Age, BMI, Gender, DC, ZT	**FLI** Age, Gender, DC, ZT

VCTE, vibration controlled transient elastography; FIB-4, Fibrosis Index Based on 4 Factors; NFS, NAFLD fibrosis score; CAP, controlled attenuation parameter; FLI, fatty liver index; BMI; DC, 'Diabetes Conventie; ZT, 'Zorg' trajectory.

RESULTS

Clinical characteristics of the T2DM study population

The final prospective observational cross-sectional study consisted of 259 T2DM **(Figure 2).** The main clinical characteristics of the study population are presented in **(Table 9).** Overall, 57.10% of the patients were male with a median age of 62 (56.00-69.00 years). Furthermore, as expected, the results revealed that the majority of the patients had significant weight problems, as 30.90% of the population was overweight, and an alarming rate of 64.10% of patients were obese with a median BMI of 31.77 kg/m^2 (28.76-35.54). Additionally, the median waist circumference of the patients was 107.75 (98.00-117.38). Moreover, 60.20% of the patients accounted for active smokers and ex-smokers, whereas most had no alcohol consumption (62.20%). Outstandingly, more than half of the study population also had an additional burden of MetS (79.90%). In line with a problematic rate of overweight and obese patients, 95.40% had a high waist circumference. Most patients had AHT (62.20%) and an uncontrolled fat metabolism as 66.00% of the patients suffered from abnormal TG values and 54.40% had low HDL values. The study population also presented broad medical history and comorbidities as a small proportion experienced a heart attack or a myocardial infarction (7.30%), suffered from cancer (10.00%), colon polyps (7.30%), underwent bariatric surgery (5.00%), and had Polycystic Ovarium Syndrome (PCOS) (2.70%). Only a small part had kidney disease (7.40%). Most of the study population suffered from obstructive sleep apnea as comorbidity (15.80%). Furthermore, several normal to abnormal laboratory results were documented. The median values for AST, ALT and GGT were respectively 27.00 (20.00-35.00) (U/L), 29.00 (20.00-46.25) (U/L), and 41.00 (23.00-82.50), revealing no severe abnormalities. Remarkably, the median Hb1Ac value of the study population was 7.10% (6.40-7.82). Lastly, in line with major weight problems, the patient's fat metabolism was clearly disturbed as the median LDL values were 63.50 mg/dL (48.75-90.00), and the median triglyceride values consisted of 161 mg/dL (106.00-231.00).

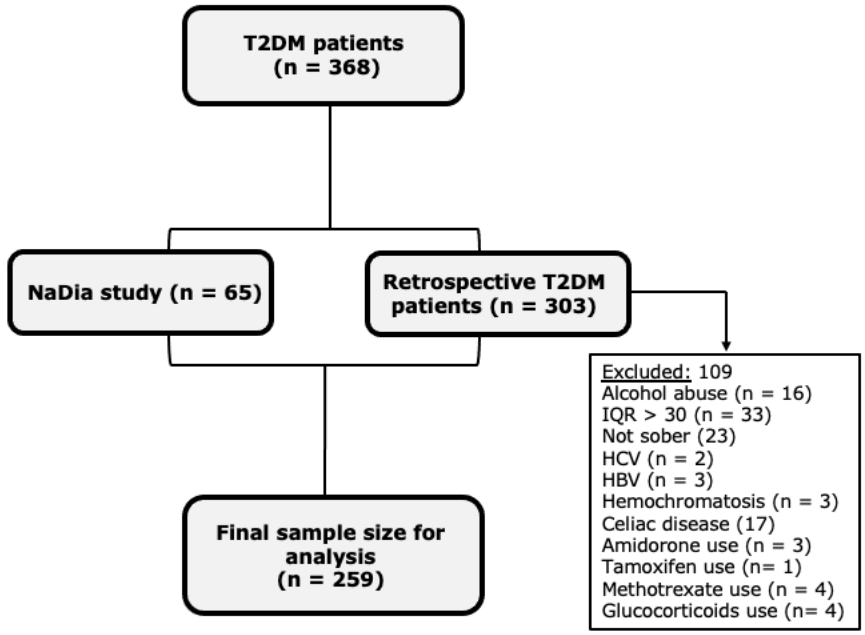

Figure 2. Flow chart of the study population.
IQR, interquartile range; HCV, Hepatitis C; HBV, Hepatitis B; T2DM, Diabetes Mellitus type 2.

Table 9. Clinical characteristics of the study population.

Demographics	Prevalence (n = 259)
Gender	
Male	148 (57.10%)
Age (years)	62.00 (56.00-69.00)
Clinical characteristics	
BMI (kg/m^2)	31.77 (28.76-35.54)
Normal	13 (5.00 %)
Overweight	80 (30.90 %)
Obese	166 (64.10 %)
Waist circumference (cms)	107.75 (98.00-117.38)
Systolic blood pressure (mmHg)	140.87 (±20.13)
Diastolic blood pressure (mmHg)	82.50 (75.00-91.00)
Alcohol	
Alcohol use	79 (30.50%)
No alcohol	161 (62.20%)
Ex-drinker	19 (7.30%)
Smoking state	
Ever smoked	151 (60.20%)
Never smoked	103 (39.80%)
Metabolic syndrome	
High waist circumference	247 (95.40%)
AHT or treatment for AHT	161 (62.20%)
FG > 100 mg/dl or T2DM diagnosis	216 (83.40%)
TG > 100 mg/dl or treatment for dyslipidemia	171 (66.00%)
HDL cholesterol < 40/50 (male/female) or treatment for dyslipidemia	141 (54.40%)

BMI, body mass index; AHT, arterial hypertension; FG, fasting glucose; TG, triglycerides; HDL, high density lipoprotein; kg/m^2, kilograms per square meter; cms, centimeters; mmHg, millimeters of mercury.

Table 9. Clinical characteristics of the study population continued.

Demographics	Prevalence (n = 259)
Comorbidities	
Cardiovascular	
MI (n = 254)	19 (7.30%)
STEMI (n = 254)	5 (1.90%)
NSTEMI (n = 252)	6 (2.30%)
Peripheral arterial disease (n = 127)	34 (13.10%)
Cancer (n = 254)	
Colon cancer	6 (2.30%)
Different type of cancer	26 (10.00%)
Gastro-intestinal (n = 252)	
Colon polyps	19 (7.30%)
Bariatric surgery	(5.00%)
Kidney disease	
Acute kidney insufficiency	10 (3.90%)
Chronic kidney insufficiency	9 (3.50%)
Pneumonology disease	
Chronic Obstructive Pulmonary Disease	4 (1.50%)
Obstructive sleep apnea (n = 185)	41 (15.80%)
Endocrinologic disease	
Polycystic ovary syndrome	7 (2.70 %)
Laboratory data	
Liver function	
AST (U/L)	27.00 (20.00-35.00)
ALT (U/L)	29.00 (20.00-46.25)
GGT (U/L)	41.00 (23.00-82.50)
Fat metabolism	
Total cholesterol (mg/dL)	145.00 (124.00-174.00)
HDL (mg/dL)	44.00 (37.00-50.00)
LDL (mg/dL)	63.50 (48.75-90.00)
Triglycerides (mg/dL)	161.00 (106.00-231.00)
Glucose metabolism	
Glucose fresh	130.50 (103.50- 175.75)
Fasting glucose	149.81(±45.90)
HbA1c (%)	7.10 (6.40-7.82)
Protein metabolism	
Total protein (g/L)	74.15 (71.48-79.15)
Albumin (g/L)	44.59 (41.65-46.89)
Gamma globulin (g/L)	11.40 (9.10-13.53)
Coagulation	
Thrombocytes (*10^9/L)	217.00(179.00-278.00)
PT (%)	96.00 (85.75-100.00)
PT (INR)	1.02 (1.00-1.10)
Iron metabolism	
Iron (µg/dL)	78.00 (60.50-97.00)
Ferritin (µg/dL)	104.00 (54.15-215.85)
Creatinine metabolism	
Serum creatinine (mg/dL)	0.89 (0.77-1.13)
eGFR (ml/min/1.73 m^2)	78.00 (64.00-90.00)
eGFR < 90(ml/min/1.73 m^2) (n=215)	139 (53.70%)

BMI, body mass index; MI, myocardial infarction; STEMI, ST-elevation MI; NSTEMI, non-ST elevation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, Hemoglobin A1c; PT, prothrombin time; INR, international normalized ratio; eGFR, estimated glomerulus filtration rate; mg/dL, milligrams per deciliters; g/L, grams per liters; U/L, units per liters.

T2DM-specific characteristics

T2DM-specific characteristics are shown in **(Table 10)**. T2DM patients were divided according to their care trajectory, answering their therapy and follow-up needs. Most of the study population had a 'Zorg' trajectory statute (39.00%) followed by 38.60% of the study population present in the 'Diabetes Conventie' trajectory. The smallest part belonged to the 'Prediabetes' trajectory (22.40%). As for T2DM-specific complications, notably, 76.40% of the patients had no complications, whereas from the patients that did have T2DM-specific complications, 14.30% of the patients had retinopathy, 5.80% had nephropathy, and another small part suffered from neuropathy (5.40%). Furthermore, extensive information was gathered on the used therapies. The primary therapies were insulin therapy and oral antidiabetics. The most used oral antidiabetic medications were GLP-1 analogues (30.10%) and biguanides (69.10%). The main insulin types injected were short-working insulin with a median of 26.00 units (4.50-74.90) and long-working insulin with a median of 28.00 units (14.00-48.75). No combination working units were used. Lastly, the main non-T2DM-specific medications used were AHT treatment consisting of beta-blockers (41.70%) and ACE-inhibitors (38.20%), and dyslipidemia treatment, majorly consisting of statin use (67.60%).

NAFLD characteristics of the T2DM study population

The main NAFLD characteristics are presented in **(Table 11).** T2DM being a significant risk population for NAFLD, most patients had steatosis according to the CAP value with a median of 314.00 (274-357.00) and the FLI with a median value of 89.14 (71.33-96.61). Only 6.20% of the T2DM patients had no NAFLD, whereas the largest part of the study population suffered from severe steatosis (61.00%). Most of the patients had no to slight fibrosis according to the BASL guidelines (57.10%) and BAVENO VII guidelines (72.20%). These results were also confirmed by the FIB-4 score (42.10%) and the NFS-score (7.70%), indicating a low risk of advanced fibrosis. However, the remaining part of the study population did show alarming rates of advanced fibrosis or cirrhosis. According to the BASL guidelines, 8.90% of the patients were classified in a significant fibrosis stage, and a larger part of the patients suffered from severe fibrosis or had characteristics suggestive of cirrhosis (34.00%). However, according to the BAVENO VII guidelines, more patients had significant fibrosis (13.10%), whereas a smaller part had a severe fibrosis stage or cirrhosis (14.70%). Similar results were seen for the FIB-4 score, demonstrating an intermediate risk of advanced fibrosis (15.80%) and a high risk of advanced fibrosis (7.70%). In addition, the NFS score also showed that, respectively 10.00% and 8.90% of the patients suffered from an intermediate risk of advanced fibrosis and a high risk of advanced fibrosis. Lastly, according to the FAST score, 11.2% of T2DM patients have NASH. However, there was no confirmation to rule in or rule out NASH in 18.1% of the patients.

Table 10.T2DM-specific characteristics of the study population.

Population characteristics	Prevalence (%) (n= 259)
Years of T2DM	9.00 (4.00-16.00)
Care trajectory	
'Diabetes Conventie'	100 (38.60%)
'Zorg' trajectory	101 (39.00%)
'Prediabetes' trajectory	58 (22.40%)
Complications (n = 61)	
No complications	198 (76.40%)
Retinopathy	37 (14.30%)
Nephropathy	15 (5.80%)
Neuropathy	14 (5.40%)
T2DM medication	
Oral antidiabetics	
GLP1-analogues (e.g., Ozempic)	78 (30.10%)
Biguanides (e.g., Metformin)	179 (69.10%)
Sulfonylurea (e.g., Uni Diamicron)	58 (22.40%)
Glinids (e.g., Repaglinid)	3 (1.20%)

T2DM, Diabetes Mellitus type 2; GLP-1, glucagon-like peptide 1.

Table 10. T2DM-specific characteristics of the study population continued.

Population characteristics	Prevalence (n = 259)
DPP-4 inhibitor (e.g., Januvia)	15 (5.80%)
SGLT-2 receptor inhibitor (e.g., Forxiga)	35 (13.50%)
Anti-neuropathy (e.g., Lyrica)	12 (4.60%)
Insulin therapy (n = 136)	136 (47.50%)
Short working	96 (37.10%)
Medium working	4 (1.50%)
Long working	110 (42.50%)
Combination	19 (7.30%)
Number of injections	4.00 (2.00-4.00)
Short-working units	26.00 (4.50 – 74.90)
Medium/long-working units	28.00 (14.00 – 48.75)
Combination-working units	0.00
Total amount of units	59.50 (14.00-48.75)
Combination therapy	
Gliflozin + gliptin (e.g., Steglujan)	2 (0.80%)
Metformin + gliptin (e.g., Eucreas)	7 (2.70%)
Metformin + glifozin	16 (6.20%)
Insulin + GLP-analogue (e.g., Xultophy)	4 (1.50%)
Other medication	35 (13.50%)
ATH treatment	136 (47.50%)
Beta blockers (e.g., Bisoprolol) (n =136)	108 (41.70%)
ACE-inhibitors (e.g., Coversyl)	99 (38.20%)
Dyslipidemia treatment	
Statins (e.g., Lipitor)	175 (67.60%)
Selective cholesterol absorption inhibitor (Ezetimbe)	16 (6.20%)
Fibrates (e.g., Lipanthyl)	12 (4.60%)
Combination lipid-lowering medication (e.g., Atozet)	17 (6.60%)

T2DM, Diabetes Mellitus type 2; GLP, glucagon-like peptide; DPP-4, dipeptidyl peptidase 4; SGLT2, sodium glucose cotransporter 2; ACE, angiotensin converting enzyme; e.g, example given.

Table 11.NAFLD characteristics of the study population.

Demographics	Prevalence (n= 259)
NAFLD characteristics	
Steatosis	
CAP	314.00 (274.00-357.00)
S0 (< 215 dB/m)	16 (6.20 %)
S1 (215 - 252 dB/m)	29 (11.20 %)
S2 (252 – 296 dB/m)	54 (20.80 %)
S3 (>296 dB/m)	158 (61.00 %)
FLI (n = 155)	89.14 (71.33-96.61)
Rule out	9.00 (3.50%)
Gray zone	17 (6.60%)
Rule in	146.00 (56.40 %)
Fibrosis	
VCTE™ (BASL)	6.60 (5.00-10.70)
F0-1	148 (57.10%)
F2-3	23 (8.90%)
F4	88 (34.00 %)
VCTE™ (BAVENO VII)	
F0-1	187 (72.20%)
F2-3	34 (13.10%)
F4	38 (14.70%)
FIB-4 (n = 170)	1.30 (0.91-1.89)
Low risk AF	109.00 (42.10%)
Intermediate risk AF	41.00 (15.80%)
High risk AF	20.00 (7.70%)
NFS (n = 69)	0.029 (±1.54)
Low risk AF	20.00 (7.70%)
Intermediate risk AF	26.00 (10.0%)
High risk AF	23 (8.90%)
NASH	
FAST (n = 185)	0.27 (0.11-0.53)
Rule in	29.00 (11.20%)
Gray zone	47.00 (18.10%)
Rule out	109.00 (42.10%)

NAFLD, non-alcoholic fatty liver disease; CAP, controlled attenuation parameter; S, steatosis; FLI, fatty liver index; VCTE, vibration controlled transient elastography; FIB-4, Fibrosis Index Based on 4 Factors; NFS, NAFLD fibrosis score; NASH, non-alcoholic steatohepatitis; FAST, FibroScan-aspartate aminotransferase.

GGT enzyme and cholesterol as liver stiffness characteristics

Several multiple regression models were tested to assess specific characteristics and risk factors in this study population. Model 1 was run to predict the association between LSM defined in VCTE values and ALT, GGT, and components of the fat metabolism (cholesterol, HDL cholesterol, LDL cholesterol, triglycerides) **(Table 12).** The model showed a significant, slightly positive association between VCTE and liver enzyme GGT (per unit, 0.31 [0.19;0.44]). Namely, higher values of this enzyme are linked to a significant increase of VCTE values **(Table**

12). In addition, cholesterol was significantly negatively associated with the VCTE value (per unit, -1.08 [-1.95;-0.22]). In contrast, no significant association was seen between the VCTE value and triglycerides, HDL cholesterol, and LDL cholesterol. Furthermore, model 2 was run to predict the association between the risk of advanced fibrosis defined in FIB-4 and liver enzyme GGT and components of the fat metabolism **(Table 12).** The model revealed a significant, slightly positive association between the FIB-4 value and GGT (per unit, 0.14 [0.034; 0.25]). On the other hand, a minor negative association was seen between the FIB-4 value and LDL cholesterol (per unit, -0.004 [-0.007; 0.00]). No significant association was found between the FIB-4 value and cholesterol, HDL cholesterol and TG. Model 3 also showed no significant association between the NFS and GGT and components of the fat metabolism **(Supplemental table 1).**

Triglycerides and ferritin as signs of steatosis

Model 4 was run to predict an association between steatosis, defined as CAP, and liver enzymes ALT, GGT, and triglycerides **(Table 13).** The model showed a significant positive association between CAP and TG (per unit, 43.97 [9.38;78.57]). However, no significant association was found between the CAP value and liver enzymes ALT and GGT. To investigate if an association was present between steatosis and HDL and ferritin, model 6 was run **(Table 13).** The model indicated a significant, slightly positive association between the FLI and ferritin (per unit, 0.026 [0.005;0.05]). No significant association was found between the FLI and HDL cholesterol. Contrarily, no significant association was found between the CAP value and ferritin **(Supplemental table 2).**

Impact of BMI, T2DM-specific medication and statin use on liver stiffness.

The risk factor models for medication use are presented to assess possible protective effects **(Table 14).** Model 1 assessed whether there is an association between the VCTE value and age, gender, BMI, insulin therapy, DPP-4 inhibitor use, SGLT-2 receptor inhibitor use, GLP-1 analogue use, and statin intake. A significant, slightly positive association was found between the VCTE value and age and BMI (per unit, 0.005 [0.002;0.009]) and (per unit, 0.001[0.003;0.016]). No significant association was found between the VCTE value, and any medication use or gender. Nevertheless, a slight negative association was seen between the VCTE value and insulin therapy and DPP-4 inhibitor use. In addition, a slight increase in VCTE values is demonstrated for females compared to males. Furthermore, model 2 showed a significant minor negative association between the FIB-4 and BMI (per unit, -0.02[-0.02; -0.002]). Correspondingly to model 1, no significant association was found between FIB-4 and any other medication use or gender. However, a slight negative association was present between the FIB-4 score and SGLT-2 receptor inhibitor use, GLP-1 analogue use, and statin use. Contrarily, model 3 showed a significant, slightly positive association between the NFS score and insulin therapy (per unit, 0.86 [0.11; 1.60]. No other significant associations were found. Nonetheless, a slight negative association was demonstrated between the NFS score and GLP-1 analogue use.

Table 12. Characteristic models liver stiffness: Blood parameters**.**

VCTE	**B-coefficient**	**95% CI for B**	**P-value**	**R^2**
		LL - UL		
Model 1			<0.001*	0.477
ALT	-0.09	-0.26 – 0.085	0.32	
GGT	0.31	0.19 – 0.44	<0.001*	
Cholesterol	-1.04	-1.89 – -0.19	0.02*	
HDL	-0.001	-0.005 – 0.004	0.79	
LDL	0.18	-0.17 – 0.52	0.31	
Triglycerides	0.05	-0.24 – 0.34	0.74	
FIB-4	**B-coefficient**	**95% CI for B**	**P-value**	**R^2**
		LL - UL		
Model 2			0.002*	0.370
GGT	0.14	0.027 – 0.25	0.02*	
Cholesterol	0.67	-0.57 – 1.91	0.29	
HDL	-0.001	- 0.006 – 0.004	0.65	
LDL	-0.004	-0.007 – 0.00	0.03*	
Triglycerides	-0.001	- 0.002 – 0.00	0.15	

VCTE, vibration controlled transient elastography; FIB-4, Fibrosis Index Based on 4 Factors; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; HDL, high density lipoprotein; LDL, low density lipoprotein; LL, lower bound; UL, upper bound; CI, confidence interval. * is considered as significant (p-value < 0.05).

Table 13. Characteristic models liver steatosis. Blood parameters.

CAP	B-coefficient	95% CI for B	P-value	R^2
		LL - UL		
Model 4			0.05*	0.277
ALT	13.56	-20.74 – 47.86	0.44	
GGT	15.94	-9.19 – 41.07	0.21	
Triglycerides	43.97	9.38 – 78.57	0.01*	
FLI	**B-coefficient**	**95% CI for B**	**P-value**	**R^2**
		LL - UL		
Model 6			0.049*	0.218
HDL	-0.053	-0.24 – 0.14	0.58	
Ferritin	0.026	0.005 – 0.05	0.016*	

CAP, controlled attenuation parameter; FLI, fatty liver index; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; HDL, high density lipoprotein; LL, lower bound; UL, upper bound; CI, confidence interval. * is considered as significant (p-value < 0.05).

Insulin therapy may control steatosis levels.

Risk model 4 was run to assess the association between steatosis defined as CAP and age, BMI, gender, insulin therapy, DPP-4 inhibitor use, SGLT-2 receptor inhibitor use, GLP-1 analogue use, and statin intake **(Table 14).** This model predicted a significant positive association between the CAP value and BMI (per unit, 5.16 [3.93;6.39]). Namely, an increase in BMI with one unit is linked with an increase of 5.16 in CAP values. Remarkably, a significant negative association was found between the CAP value and the use of insulin therapy (per unit, -16.13 [-29.33;2.93]). Thereby, insulin therapy is associated with a decrease in CAP value. No significant association was found between CAP value and any other medication or gender. However, the model showed a reduction in CAP values for females compared to males. This model was also tested with the FLI where no significant associations were found **(Supplemental table 3).**

Metabolic syndrome is a significant risk factor for steatosis levels

Risk factor model 5 tested whether there is an association between steatosis defined as FLI and age, gender, HbA1c, and MetS **(Table 15).** This model demonstrated a significant positive association between the FLI and the presence of MetS (per unit, 21.48 [7.46;35.71]). Namely, the presence of MetS increases the FLI value, confirming that the presence of MetS is a major risk factor for steatosis. No other significant association was found in this model. However, a positive association was demonstrated between the FLI and HbA1c. This model was also tested for VCTE, FIB-4, NFS, and CAP, but no significant association was found for HbA1c and MetS **(Supplemental table 4).**

The impact of care trajectories on liver stiffness

Risk model 1 investigated whether there is an association between the VCTE value and age, gender, BMI, and the care trajectories 'Diabetes Conventie' trajectory and 'Zorg' trajectory **(Supplemental table 5).** This model demonstrated a significant, slightly positive association between the VCTE value and age and BMI (per unit, 0.006 [0.002;0.009]) and (per unit 0.010 [0.003;0.02]). No other significant associations were found. Nonetheless, a slightly negative association between the VCTE value and 'Zorg' trajectory and 'Diabetes Convention' trajectory was seen in comparison to the 'Prediabetes' trajectory. This model was also tested for FIB-4, NFS, CAP and FLI. However, no significant associations were found **(Supplemental table 5).**

Table 14.Risk models for liver stiffness and steatosis: Medication use.

VCTE	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 1			0.028*	0.257
Age	0.005	0.002 – 0.009	0.005*	
BMI	0.010	0.003 – 0.016	0.006*	
Gender	0.056	-0.02 – 0.13	0.15	
Insulin therapy	-0.016	-0.09 – 0.6	0.66	
DPP-4 inhibitor	-0.033	-0.19 – 0.13	0.68	
SGLT-2 RI	0.014	-0.093 – 0.12	0.80	
GLP-1	0.002	-0.08 -0.84	0.96	
Statins	0.001	-0.8 – 0.08	0.97	
FIB-4	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 2			0.046*	0.286
Gender	-0.15	-0.067 – -0.053	0.72	
BMI	-0.010	-0.02 – -0.002	0.014*	
Insulin therapy	0.057	-0.024 – 0.14	0.17	
DPP-4 inhibitor	0.030	-0.16 – 0.21	0.79	
SGLT-2 RI	-0.03	-0.14 – 0.085	0.62	
GLP-1	-0.91	-0.18 – 0.00	0.51	
Statins	-0.001	-0.90 – 0.90	0.98	
NFS	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 3			0.10	0.384
Gender	-0.13	-0.84 – 0.59	0.72	
Insulin therapy	0.86	0.11 – 1.60	0.025*	
DPP-4 inhibitor	0.58	-1.61 – 2.78	0.60	
SGLT-2 RI	-0.60	-1.78 – 0.59	0.32	
GLP-1	-0.76	-1.61 – 0.058	0.07	
Statins	-0.11	-0.89 – 0.67	0.78	
CAP	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 4			<0.001*	0.508
Age	-0.20	-0.84 – 0.44	0.537	
BMI	5.16	3.93 – 6.39	<0.001*	
Gender	-8.86	-22.15 – 4.44	0.19	
Insulin therapy	-16.13	-29.33 – 2.93	0.017*	
DPP-4 inhibitor	-9.007	-37.20 – 19.18	0.53	
SGLT-2 RI	6.36	-12.79 – 25.52	0.51	
GLP-1	4.91	-9.72 – 19.54	0.51	
Statins	-10.73	-24.65 – 3.20	0.13	

VCTE, Vibration controlled transient elastography; NFS, NAFLD fibrosis score; FIB-4, Fibrosis Index Based on 4 Factors; CAP, Controlled attenuation parameter; BMI, body mass index; DC, Diabetes Convention; ZT, Zorg trajectory; DPP-4, dipeptidyl peptidase-4; SGLT2, sodium glucose cotransporter-2; GLP-1, glucagon-like peptide 1; RI, receptor inhibitor; LL, lower bound; UL, upper bound; CI, confidence interval. * is considered as significant (p-value < 0.05).

Table 15. Risk model for liver steatosis. HbA1c and MetS.

FLI	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 5			0.003*	0.337
Age	-0.35	-0.70 – 0.001	0.51	
Gender	-0.40	-8.25 – 7.44	0.92	
HbA1c	2.75	-0.048 – 5.55	0.054	
MetS	21.48	7.46 – 35.71	0.003*	

FLI, fatty liver index; BMI, body mass index; HbA1c, Hemoglobin A1c; MetS, metabolic syndrome; lower bound; UL, upper bound; CI, confidence interval. * is considered as significant (p-value < 0.05).

DISCUSSION

This cross-sectional study aimed to profile Belgian T2DM patients followed in secondary care to identify specific risk factors for developing NAFLD. Therefore, the prevalence of NAFLD in this study population was 93.00%, according to the FibroScan® measurements. During the past ten years, the prevalence of NAFLD has increased rapidly, simultaneously with rising obesity and T2DM prevalence rates (37, 34). Also, most of these patients were obese (61.00%). The study findings strongly confirm that T2DM and obesity are major risk factors for NAFLD (46). Similar to our study, *Toi Chu et al*. established a high prevalence of NAFLD in T2DM patients based on the FibroScan® (73.30%) (n = 225) (47). Literature demonstrates that subjects with T2DM have an increased risk of developing NASH, advanced fibrosis, cirrhosis, and even HCC (18). According to the BASL and BAVENO VII guidelines, the smallest part of our study population, respectively had moderate fibrosis (8.90 % and 13.10 %) and severe fibrosis or cirrhosis (34.00 % and 14.70 %). The study's findings show that the largest part of the T2DM patients had no fibrosis according to the VCTE measurement (72.20 %) and a low risk of advanced fibrosis based on the FIB-4 (42.10 %) and NFS scores (7.70%). Another large cohort with T2DM patients also showed that the majority of the patients had no or mild fibrosis (72.60%), and a smaller part suffered from moderate to significant fibrosis (19.60%) and severe fibrosis or cirrhosis (8.20%) (n = 534) (48). Although not everyone had fibrosis, those who have it can develop complications. Therefore, these findings suggest that any prevalence of hepatic fibrosis requires increased vigilance, thorough follow-up, and corrective lifestyle changes before patients reach irreversible NAFLD stages, especially in this risk population (47, 49)

Liver stiffness and steatosis characteristics were evaluated to characterize NAFLD thoroughly. According to our regression models, significant features of NAFLD in T2DM patients were liver enzyme GGT and LDL cholesterol for liver stiffness and GGT and triglycerides for liver steatosis. To screen NAFLD, liver enzymes ALT and GGT are often used as the first clinical biochemical indicators of injured hepatocytes (50). Namely, NAFLD is typically characterized by mild elevations or 1-2 times the upper limit of normal (50, 51). Despite having NAFLD, up to 50 % of NAFLD patients can have normal liver enzyme levels (50). The study results show that liver enzyme GGT may play a role as an essential characteristic in both liver stiffness and steatosis. However, the association was not present for steatosis. Moreover, the median value of liver enzymes in our study population also appeared normal. According to a prior study (n = 515) in a biopsy-proven NAFLD cohort, individuals with normal or abnormal transaminase levels had a similar prevalence of advanced fibrosis (51). Another cross-sectional study demonstrated that recently diagnosed T2DM subjects with NAFLD had significantly higher liver enzyme values (n = 158) (52). *Nurshad A. et al*. also found that increased GGT levels were independently associated with T2DM in patients with NAFLD (53). One of the possible mechanisms linking liver enzymes to T2DM is liver inflammation through increasing pro-inflammatory reactions (54). GGT is essential in the metabolism and synthesis of extracellular glutathione, a key antioxidant in the body's defense mechanisms (55). Therefore, increased GGT activity might increase glutathione consumption (56). Hence, increased GGT activity in these patients may reflect oxidative stress. Oxidative stress is also linked to insulin resistance, making GGT a marker of oxidative stress, especially in T2DM patients suffering from NAFLD (55, 56).

The study findings further confirm that the involvement of fat metabolism is significantly associated with NAFLD in these patients. Namely, TG levels are significantly associated with steatosis defined by CAP in T2DM patients. *Zhou Q. et al.* demonstrated that TG levels between T2DM patients with and without NAFLD differed substantially in T2DM patients with NAFLD. Moreover, they also showed that high levels of TG remained a highly significant predictor of NAFLD (57). Disease development of NAFLD is defined by the accumulation of fat in the form of TG in hepatocytes (58, 59, 60). Therefore, hepatic steatosis results from increased unmetabolized energy in hepatocytes exceeding the energy combustion capability of fatty acid oxidation mechanisms (61). The increased uptake of dietary free fatty acids, increased TG synthesis in the liver (de novo lipogenesis), decreased TG hydrolysis, and fatty acid beta-oxidation are described as mechanisms linking TG to NAFLD (59, 62). Another important pathophysiological factor is IR. The storage of TG in the liver is increased through lipolysis and de novo lipogenesis induced by IR (63). Furthermore, the study results show a significant negative association between LDL cholesterol and NAFLD. It has been shown that lowering LDL cholesterol is crucial for treating dyslipidemia in patients with NAFLD. The most recommended medications are statins since they play a fixed role in the primary and secondary prevention of CVD. Moreover, evidence also shows that statins may improve NASH stages and have protective effects on advanced liver fibrosis in T2DM patients (64, 65). It is also important to mention that we found a significant negative association between total cholesterol levels and NAFLD. This

finding contrasts existing literature since sufficient evidence demonstrates that the pathogenesis of NAFLD is associated with the accumulation of free cholesterol and the imbalance of cholesterol homeostasis in the liver (66). When interpreting this result, we must remember that most of the study population has active statin use, lowering LDL-cholesterol and thereby total cholesterol, which may have interfered with this outcome (67).

Our last characteristic model showed a significant positive correlation between serum ferritin levels and NAFLD defined in the FLI. Serum ferritin has also emerged as a potential indicator for NAFLD (68). Several studies have demonstrated that one-third of individuals with NAFLD have an iron overload. This excess rises from steatosis, IR, and inflammation, causing an altered regulation of iron transport (69). *Chang M-O et al.* also showed a significant association between serum ferritin levels and NAFLD (n = 25.597) (70).

The main risk factors in our study population were the BMI and the presence of MetS. Over the past few decades, global changes in dietary and exercise habits, often tied to growing urbanization, have fueled an epidemic rise in obesity and T2DM and, therefore, NAFLD (71, 72, 73). *Toi Chu et al.* also found, in line with the study findings, that T2DM patients with NAFLD had a higher BMI and were obese (47). Another cross-sectional study performed in Amman, Jordan, showed that overweight, diabetic patients and obese diabetic patients had 2.72 times and 4.77 times higher risk of developing NAFLD than patients with a normal BMI. (74). Hence, a higher BMI increases the likelihood of NAFLD development and presence among T2DM patients. This significant association between a high BMI and NAFLD can be explained by the pathophysiological factor in both T2DM and NAFLD, namely IR (15, 74, 75). Adipose tissue in obese individuals generates increased levels of non-esterified fatty acids, glycerol, hormones, and pro-inflammatory cytokines that may contribute to the development of IR (75).

No pharmacological treatment for NAFLD has been approved by international authorities for NAFLD, regardless of the presence or absence of T2DM. As mentioned above, lifestyle modifications are essential in the clinical management of NAFLD across the disease spectrum (39). Given the close relationship between NAFLD, various anti-diabetic medications have been tested. These anti-diabetics may benefit the NAFLD spectrum, some of which may even prevent non-liver-related cardiovascular outcomes (39, 76). Therefore, we investigated the association between specific anti-diabetic medications, statin use and liver stiffness/steatosis, which have been proven to have possible beneficial effects on the disease. In the study population, surprisingly, the use of insulin therapy was strongly and significantly negatively correlated with steatosis levels defined in CAP. This means that when one individual is treated with insulin, a decrease of 16.13 units is present in the CAP value. In a comparative study of insulin therapy and oral antidiabetics, *Marc E. et al*. found that patients treated with insulin for at least three years showed a significant reduction in steatosis compared to patients treated with oral anti-diabetics (n = 94) (77). Another study where Cox regression models were performed, found that insulin, as an anabolic hormone, also reduced the risk of NAFLD (78). Furthermore, other prospective studies have demonstrated that treatment with various insulin types can improve liver fat content (79, 80, 81). Moreover, it was unusual in these studies that the addition of insulin therapy was not associated with significant weight gain among the subjects (82). While weight gain is a side effect of insulin therapy, the amount can differ between insulin types and dosages (83). Notwithstanding, in T2DM patients, BMI increase is correlated with treatment intensity during the first year of treatment (84). Insulin therapy has also been recommended in T2DM patients with liver cirrhosis or advanced liver dysfunction (85). The need may be higher in patients with compensated cirrhosis compared to decompensated patients due to a reduction in hepatic clearance and gluconeogenesis (85, 86). Even though some evidence shows improvement in NAFLD, insulin therapy has also been associated with weight gain and an increased risk of cardiovascular events, which are key risk factors and outcomes within NAFLD itself (39, 87). The differences between the study results can be explained by the need for intensive insulin therapy or a strict glycemic control. Namely, significant weight gain is primarily present in T2DM patients commencing intensive insulin therapy (88). Thus, in our study population, the significant negative association between insulin therapy and steatosis might be related to a more controlled, long-term treatment with insulin rather than an intensive treatment. Nevertheless, the longitudinal impact of glycemic control managed by insulin therapy among T2DM patients suffering from NAFLD is not widely available (82, 89). Several possible mechanisms have been described by which glycemic control might improve NAFLD. In patients with NAFLD and T2DM, glucose is the substrate for de novo lipogenesis, forming a key contribution to hepatic TG accumulation. This means that enhancing glycemic control would limit the presence of glucose as a substrate for hepatic de novo lipogenesis. Additionally, glucose can activate stellate cells, potentially modulating the

fibrotic response to lipid and inflammatory insults, the main drivers of advanced NAFLD (82).

In contrast with existing evidence, we found several non-significant negative associations between the use of anti-diabetics, statin use, and NAFLD. Anti-diabetics inducing weight loss, known SGLT-2 receptor inhibitors and GLP-1 analogues, have been demonstrated to have protective effects of NAFLD. Several studies have shown that both therapies can induce a reduction of liver fat content (64, 76, 78). Moreover, *Perseghin G. et al.* showed a protective effect of statin use on advanced fibrosis in T2DM patients (64). Similar evidence also states that statin use may also enhance the NASH disease stage and lower the risk of liver fibrosis (65).

Nevertheless, insufficient evidence and even conflicting data are available regarding the impact of various anti-diabetics on the emergence of NAFLD. In addition, the pathophysiology of NAFLD and T2DM remains complex and poorly understood. Even though our analysis is limited in its ability to explain certain associations and mechanisms, our findings should encourage more functional research in this area to elucidate this complex relationship.

LIMITATIONS

When reporting our findings, certain limitations of our study should be recognized. The main limitation of this cross-sectional study was that the diagnosis of NAFLD was not based on the gold standard liver biopsy, but rather on a combination of FibroScan® measurements and noninvasive test scores. Additionally, missing data was present in the medical patient records, limiting some of our outcomes, such as calculating noninvasive test scores. Our recruited subjects were sampled from one hospital, which makes our subjects prone to selection bias. Furthermore, the subjects were directly sent to us by endocrinologists, limiting their representativeness for the general T2DM patient population and explaining the high prevalence of NAFLD in our population. On top of that, our sample size was relatively small, explaining some of the unusual findings and non-significant results. The study set-up was cross-sectional, limiting the temporal link between the outcome and exposure. Additionally, ongoing, and continuously changing guidelines to interpret NAFLD diagnosis based on the LSM and CAP value and the noninvasive test scores, limit the generalization of NAFLD diagnosis globally. This study also had no control group since only a few T2DM subjects without NAFLD were present. Future research is required to validate our findings in larger sample sizes that are representative of the general population. Future research can also focus on comparing the risk factors between NAFLD and non-NAFLD groups within T2DM patients.

CONCLUSION

To the best of our knowledge, we are the first to compare different NAFLD diagnosis strategies in Belgium, studying the specific characteristics and risk factors of NAFLD in T2DM patients. Our findings strongly emphasize the complex link between NAFLD and T2DM. Particular characteristics of NAFLD in T2DM patients have been found: liver enzyme GGT and components of the fat metabolism, LDL-cholesterol, and TG. Furthermore, the main risk factors were BMI and the presence of MetS. These results emphasize the alarming need for united NAFLD management and treatment strategies, not only in the general population, but also in specific risk populations such as T2DM. The data regarding treatment with anti-diabetics to control NAFLD remains insufficient and conflicting. Therefore, future research is required to clarify the link between anti-diabetics and NAFLD. We believe that these findings can form a basis for the next step to elucidate the complex relationship between T2DM and NAFLD.

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AUTHOR CONTRIBUTIONS
Mrs. L.H and Prof Dr. G.R. conceived and designed the cross-sectional study in ZOL. E.T collected and analyzed the data for this manuscript as a part of the senior's internship. All authors provided feedback on the manuscript.

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SUPPLEMENTAL TABLES

Supplemental table 1.Characteristic models liver stiffness: Blood parameters.

NFS	B-coefficient	95% CI for B		P-value	R^2
		LL	UL		
Model 3				0.037	0.470
GGT	0.004	-0.001 – 0.009		0.12	
Cholesterol	-2.50	-8.71 – 3.72		0.42	
HDL	0.52	-4.44 – 5.47		0.83	
LDL	-0.95	-3.39 – 1.50		0.44	
Triglycerides	-0.70	3.50 – 2.09		0.62	

GGT, gamma glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Supplemental table 2. Characteristic model for liver steatosis: Blood parameters.

CAP	B-coefficient	95% CI for B		P-value	R^2
		LL	UL		
Model 5				0.40	0.205
HDL	-61.70	-197.34 – 73.94		0.37	
Ferritin	0.058	-0.014 – 0.13		0.11	

CAP, controlled attenuation parameter; HDL, high-density lipoprotein; LL, lower bound; UL, upper bound; CI, confidence interval.

Supplemental table 3. Risk model liver steatosis: Medication use.

FLI	B-coefficient	95% CI for B		P-value	R^2
		LL	UL		
Model 5				0.43	0.214
Age	-2.80	-0.61 – 0.063		0.11	
Gender	-1.59	-9.36 – 6.17		0.69	
Insulin therapy	-2.66	-10.30 – 4.99		0.49	
DPP-4 inhibitor	-1.53	-2.56 – 14.03		0.85	
SGLT-2 RI	6.507	-16.97 – 13.92		0.26	
GLP-1	5.74	-2.56 – 14.029		0.17	
Statins	0.65	-7.43 – 8.73		0.87	

FLI, fatty liver index; DPP-4, dipeptidyl peptidase-4; SGLT-2, sodium glucose cotransporter-2; GLP-1, glucagon-like peptide 1; LL, lower bound; UL, upper bound; CI, confidence interval.

Supplemental table 4. Risk models for liver stiffness and steatosis. HbA1c and MetS.

VCTE	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 6			0.16	0.194
Age	0.003	-0.001 – 0.007	0.15	
BMI	0.037	-0.041 – 0.12	0.35	
Gender	0.007	0.00 – 0.014	0.049*	
HbA1c	0.002	-0.027 – 0.030	0.91	
MetS	0.054	-0.052 – 0.16	0.32	
FIB-4	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 7			0.61	0.246
Gender	-0.032	-0.12 – 0.055	0.47	
BMI	-0.012	-0.020 – -0.004	0.005*	
HbA1c	0.007	-0.023 – 0.038	0.63	
MetS	0.012	-0.11 – 0.14	0.85	
NFS	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 8			0.82	0.140
Gender	-0.34	-1.32 – 0.65	0.50	
HbA1c	-0.025	-0.46 – 0.41	0.91	
MetS	0.38	-1.19 – 1.95	0.63	
CAP	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 9			<0.001*	0.236
Age	-0.44	-1.17 – 0.29	0.24	
Gender	-6.67	-21.33 – 7.98	0.37	
BMI	5.03	3.67 – 6.39	<0.001*	
HbA1c	1.57	-3.73 – 6.87	0.56	
MetS	0.27	-19.49 – 20.04	0.98	

VCTE, vibration controlled transient elastography; FIB-4, Fibrosis Index Based on 4 Factors; NFS, NAFLD fibrosis score; CAP, controlled attenuation parameter; MetS, metabolic syndrome; HbA1c, hemoglobin A1c. * is considered as significant (p-value < 0.05).

Supplemental table 5. Risk models for liver stiffness and steatosis: Care trajectories.

VCTE	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 1			0.001*	0.277
Age	0.006	0.002 – 0.009	0.002*	
BMI	0.010	0.003 – 0.02	0.003*	
Gender	0.046	-0.027 – 0.12	0.22	
DC	-0.067	-0.16 – 0.03	0.17	
ZT	0.042	-0.089 – 0.006	0.09	
FIB-4	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 2			0.089	0.100
BMI	-0.011	-0.019 – -0.003	0.006	
Gender	-0.017	-0.099 – 0.066	0.69	
DC	0.007	-0.098 – 0.11	0.89	
ZT	-0.004	-0.055 – 0.47	0.88	
NFS	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 3			0.41	0.205
Gender	-0.010	-0.75 – 0.72	0.98	
DC	0.20	-0.72 – 1.12	0.67	
ZT	0.36	-0.068 – 0.79	0.098	
CAP	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 4			<0.001*	0.140
Age	-0.33	-0.96 – 0.30	0.31	
BMI	5.13	3.93 – 6.34	<0.001*	
Gender	-8.47	-21.69 – 4.75	0.21	
DC	-14.032	-31.48 – 3.41	0.11	
ZT	-4.56	-13.23 – 4.12	0.30	
FLI	**B-coefficient**	**95% CI for B** LL - UL	**P-value**	**R^2**
Model 5			0.094	0.226
Age	-0.26	-1.17 – 0.29	0.24	
Gender	-2.54	-21.33 – 7.98	0.37	
DC	-8.06	-3.73 – 6.87	0.56	
ZT	-5.24	-19.49 – 20.04	0.98	

VCTE, Vibration controlled transient elastography; NFS, NAFLD fibrosis score; FIB-4, Fibrosis Index Based on 4 Factors; CAP, Controlled attenuation parameter; BMI, body mass index; DC, Diabetes Convention; ZT, Zorg trajectory; LL, lower bound; UL, upper bound; CI, confidence interval. * is considered as significant (p-value < 0.05).