Noninvasive Assessment of Nonalcoholic Fatty Liver Disease in Type 2 Diabetes Patients

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ABSTRACT

Background & aims: Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder in industrialized countries. Patients with type 2 diabetes mellitus (T2DM) are at a higher risk of developing NAFLD and they are more likely to evolve to more severe disease. Until today noninvasive tools to screen and differentiate for NAFLD severity are unavailable. We aimed to get a better perspective of the distribution of NAFLD in patients with T2DM by using existing scores to screen for liver disease.

Methods: Patients with T2DM presenting at the diabetes clinic of the UZ Brussel were evaluated by noninvasive routine screening. Different scores were calculated with the obtained parameters.

Results: 44 patients (25 male, 19 female) between 37 and 65 years of age were included. All met the criteria of metabolic syndrome. 82% of them had NAFLD diagnosed by ultrasound. 9 to 50% of the patients had fibrosis depending of which score was used. Patients were more likely to have more severe disease when having elevated liver enzymes. There was no correlation with age. The correlation with insulin therapy was negative. The different tested scores were positively correlated with each other.

Conclusion: Increased awareness of the magnitude of significant liver disease in T2DM patients is necessary. The next step in our research will be performing liver biopsies and validate a score or develop a new specific score for T2DM patients.

Keywords: Nonalcoholic Fatty Liver Disease, Type 2 Diabetes Mellitus, Noninvasive Scores.

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; IDF, International Diabetes Federation; MS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus; USS, ultrasound steatosis score.
BACKGROUND & AIMS
Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder in industrialized
countries[1]. It is defined as hepatic steatosis, with or without inflammation or fibrosis in
absence of secondary hepatic fat accumulation. NAFLD comprises nonalcoholic fatty liver
(NAFL) and nonalcoholic steatohepatits (NASH). The first being defined as hepatic steatosis
without evidence of inflammation, the latter with hepatic inflammation with or without fibrosis.
Because NASH has a worse prognosis, with a risk for evolution to cirrhosis and even to
hepatocellular cancer, it is important to discriminate it from NAFL.

Risk factors for developing NAFLD include obesity, insulin resistance and metabolic syndrome
(MS). One of the major risk factors for developing NAFLD is type 2 diabetes mellitus
(T2DM)[2]. The prevalence in T2DM patients is up to 74% for NAFLD and up to 22% for
NASH[3]. The pathogenesis of NAFLD has not been fully elucidated yet. It is believed that
insulin resistance (IR) is a key mechanism, which makes the association with T2DM clear. IR is
a condition in which a given concentration of insulin does not satisfy the need to stimulate the
glucose uptake and utilization as much as it does in normal people, in the end resulting in
hyperglycemia. In the beginning, IR is still compensated by stimulating the pancreatic beta cells
to produce more insulin, in order to maintain a normal blood glucose level. However, after time,
the beta cells get exhausted which results in decreased insulin secretion, leading to
hyperglycemia. Furthermore, IR causes an increase of lipase action in adipocytes resulting in
more circulating free fatty acids (FFA). These FFAs will pass through the portal system and
accumulate in the liver. On top of this, the FFAs inhibit insulin action. This is a sign for the liver
to start making glucose by increasing gluconeogenesis and glycogenolysis. Another effect is the
stimulation of lipogenic transcription factors which stimulate the liver to synthesise fat, called de
novo lipogenesis[4]. So there is both an increase in fat imported into the liver and in liver fat
production. This process is often referred to as ‘the first hit’. Besides IR and hyperglycemia,
increased oxidative stress, inflammation and mitochondrial dysfunction contribute to the
development of both steatosis and steatohepatitis, also called ‘the second hit’. Recent studies
showed that FFA promotes this process, and that hepatic triglycerides may be protective[5].

However, not all patients with NASH have insulin resistance. It is suggested that a complex
interplay between earlier mentioned factors and underlying genetic predisposition are at the base
of the development of NAFLD. This emphasizes the need for further research.
In an era that the number of patients diagnosed with T2DM increases every day, the number of patients having NAFLD increases as well, and thus the need for a proper screening tool becomes important.

Patients with NAFLD are generally asymptomatic. Occasionally there are symptoms of fatigue or vague right upper quadrant pain. Hepatomegaly and acanthosis nigricans can be observed, but are more frequent in the pediatric population. Diabetes related variables, including glycemic control and duration of diabetes were not related to more severe stages of NAFLD[6]. However, there is more and more evidence that microvascular and macrovascular complications are associated with NAFLD[7]. Gender, age and ethnicity are also associated. The prevalence of NAFLD increases with age. Different studies reported that males are at a greater risk of developing NAFLD[8], while other studies refute this, obtaining no consensus on the influence of gender. If it comes to ethnicity, Hispanic individuals are more likely to develop NAFLD. Among the Black population, on the contrary, there is a lower prevalence[9]. Having family members with diabetes is now also suggested as a risk factor for NASH and fibrosis[10].

Laboratory tests are until today insufficient to diagnose NAFLD, nor can they differentiate between the different stages of severity. Abnormal liver enzymes may be seen in less than 20% of patients with diabetes and histologically proven NASH[11]. Moreover, it is thought that in patients with T2DM liver enzymes are less representative. A case-control study showed that liver fat content in T2DM patients was underestimated by serum alanine aminotransferase (ALT), compared to non-diabetic patients[12]. On the other hand, ALT and alkaline phosphatase (AP) levels were higher, but still within normal limits, in patients with NASH[13]. Also, patients with any stage of fibrosis had higher levels of aminotransferases and a higher aspartate aminotransferase (AST) to ALT ratio[14]. Other abnormalities frequently seen in patients with NAFLD are mildly elevated serum ferritin and autoantibodies. Both should lead to further investigation of respectively hemochromatosis and autoimmune liver disease, but are generally considered to be epiphenomena of NAFLD[15]. Other screening parameters are being investigated, but are not routinely available and are expensive, which makes them hard to use in daily practice.

Radiologic methods can detect NAFLD, but no imaging modality is able to differentiate between the histologic subtypes. Liver ultrasonography (US) is a largely available, inexpensive and a safe
way to diagnose moderate-to-severe hepatic steatosis. The grading system is based on echogenicity resulting in a four-point scale: normal (grade 0), mild (grade 1), moderate (grade 2) and severe (grade 3). It is however less accurate for detecting mild steatosis and it is operator dependent. Underlying chronic liver disease or fibrosis can also change the echogenicity, making the observer susceptible to grade incorrectly[17]. The score could be useful in selecting people for liver biopsy. Computed tomography (CT) can detect and quantify steatosis, but does not differentiate the grades of NAFLD[16].

Magnetic resonance (MR), especially MR spectroscopy, is very accurate for detecting NASH[18]. Unfortunately, MR is expensive, making it not an ideal screening tool. Another promising radiologic method is transient elastography, better known as the Fibroscan. It is an US-based technology for assessing liver stiffness. It has been validated for viral hepatitis and cholestatic liver disease, but not for patients with T2DM[19]. Another problem is its use on obese patients, because it does not penetrate easily through fat tissue, which limits its use in patients with NAFLD[19].

The gold standard to diagnose and differentiate the grade of NAFLD is a liver biopsy. However, this procedure has some morbidity and mortality, making it undesirable to perform without founded suspicion for the presence of NAFLD.

The current indications for liver biopsy are uncertain diagnosis of NAFLD after obtaining standard laboratory tests and hepatic imaging, and staging of inflammation and fibrosis. Several recent studies propose to biopsy patients who are at increased risk for advanced fibrosis or cirrhosis, including patients with T2DM[20]. Recent guidelines recommend liver biopsy in all patients with T2DM who have steatosis on liver US.

The objective of the present study is to implement available scores, used in daily practice to screen for fibrosis, onto patients with T2DM. In this way, we want to get a better perspective on the distribution of liver disease in the population with T2DM.

**PATIENTS & METHODS**
All patients with T2DM presenting at the outpatient diabetes clinic of the University Hospital, UZ Brussel, for annual follow-up, undergo standard examinations including physical
examination, blood tests and a liver US to screen for steatosis. Both the blood test and liver US are performed under fasting conditions. Results obtained from these examinations were used to calculate the selected scores. The study was approved by the ethical committee UZ Brussels (B.U.N. 143201422455).

Inclusion criteria: Female and male patients with T2DM aged between 35 and 65 years who underwent annual standard examinations in the diabetic clinic. This is based on several epidemiologic studies where older age was associated with the presence of liver disease[21]. Furthermore, the mean age for diagnosing fatty liver is around 45 years[22].

Exclusion criteria: the presence of another known cause for liver disease e.g. alcohol ingestion >20g per day for women and >30g per day for men, viral hepatitis A, B or C, human immunodeficiency virus, the use of hepatotoxic drugs (tamoxifen, amiodarone and methotrexate) and prednisolone, exposure to toxins as vinyl chloride, total parental nutrition, cachexia, intestinal bypass surgery and the presence of biliary, autoimmune or metabolic liver disease. No pregnant or breast feeding women were allowed.

Scores were selected based on recent studies and include the Antwerp NASH score 1 and 2, the Antwerp NASH severity score, the Antwerp NAFLD significant fibrosis score, the Antwerp NAFLD advanced fibrosis score[25], the FIB-4 score and the NAFLD fibrosis score. All these scores can be calculated using results from annual standard examinations.

The different Antwerp scores were developed to screen for NASH and fibrosis in an overweight and obese population. The Antwerp NASH score 1 and 2 are scores developed to predict the diagnosis of NASH according to the definition by Brunt et al and Kleiner et al respectively. Brunt et al and Kleiner et al are two definitions used to score liver biopsies for NAFLD. The Antwerp NASH score 1 contains simple laboratory parameters and a liver US and is calculated according to the following formula: -3.613 + [1.315 x (ALT >40 U/L: no = 0; yes = 1)] + [1.084 x USS] + [1.223 x fasting C-peptide (nmol/L)]. The US appearance of the liver parenchyma was scored by using a modification of the Saverymuttu[26] classification by making the sum of the echogenicity of the liver parenchyma compared with the renal parenchyma (no hyperechogenicity, 0; mild-to-moderate hyperechogenicity, 1; moderate-to-severe hyperechogenicity, 2) and posterior beam attenuation (absent, 0; present, 1), resulting in an
ultrasound steatosis score (USS) ranging from 0 to 3. In our study, one radiologist performed all US and calculated the score, to avoid interobserver variability. The Antwerp NASH score 2 to predict the diagnosis of NASH according to the definition by Kleiner et al. is calculated according to the following formula: $-6.117 + [0.029 \times ALT (U/L)] + [1.394 \times USS] + [1.223 \times number \ of \ International \ Diabetes \ Federation \ (IDF) \ criteria \ for \ metabolic \ syndrome \ (MS)]$. The criteria for MS are the following: Central obesity (defined as waist circumference $\geq 94$cm for Europid men and $\geq 80$cm for Europid women, with ethnicity specific values for other groups) plus any two of the following four (1) raised TG level: $\geq 150$ mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality (2) reduced HDL cholesterol: $< 40$ mg/dL (1.03 mmol/L) in males and $< 50$ mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality (3) raised blood pressure: systolic BP $\geq 130$ or diastolic BP $\geq 85$ mm Hg, or treatment of previously diagnosed hypertension (4) raised fasting plasma glucose (FPG) $\geq 100$ mg/dL (5.6 mmol/L), or previously diagnosed type 2. The NASH activity score comprises the same parameters as the Antwerp NASH score $1 - 0.845 + [0.021 \times ALT (U/L)] + [1.200 \times USS] + [0.936 \times fasting \ C$-peptide (nmol/L)] [27]. The outcome is correlated with the severity of NASH as expressed by the NASH Activity Score (NAS). It is calculated by making the sum of the scores for steatosis, lobular inflammation, and ballooning when looking at a liver biopsy. The Antwerp NAFLD significant fibrosis score to predict the diagnosis of significant fibrosis: $-10.593 + [0.048 \times waist (cm)] + [1.373 \times fasting \ C$-peptide (nmol/L)] + [0.043 \times AST (U/L)]. The waist was measured using the standard method. The Antwerp Advanced fibrosis score to predict the diagnosis of advanced fibrosis: $-13.376 + [0.057 \times waist (cm)] + [0.112 \times AST (U/L)] - [3.078 \times AST > 40 \ U/L: \ no = 0, \ yes = 1]$.

All the Antwerp scores use two cutoff values. If the patient scores under the first, this means he probably will not have the disease, if he scores above the second cutoff, he probably has the disease. If he falls in between, a liver biopsy is needed to make a diagnosis.

The FIB-4 score combines age with three standard biochemical values (platelets, ALT and AST) to assess fibrosis: $[Age \ (years \times AST \ (U/L)) \ / [Platelets \ (x10^9/L) \times \sqrt{ALT \ (U/L)}]$. A cutoff value of 1.3 is used. If the patient scores under, he probably does not have fibrosis, if he scores above, he probably has liver fibrosis. In NAFLD, FIB-4 has demonstrated a high area under the curve (AUROC) for the diagnosis of advanced fibrosis, but it is not capable of diagnosing NASH.
The NAFLD fibrosis score is a panel comprising six variables: age, hyperglycaemia, BMI, platelet count, albumin and AST/ALT ratio. It is calculated according to the following formula: 

$$-1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{BMI (kg/m}^2\text{)} + 1.13 \times \text{Impaired fasting glucose/diabetes (yes = 1, no = 0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet (x}10^9/L\text{)} - 0.66 \times \text{albumin (g/dL)}.$$

The calculation returns a continuous score, which can be divided into 3 groups, using the cutoff values -1,455 and 0,675. A score in between means an indeterminate result[28] and needs to be evaluated by a biopsy.

Both the FIB-4 and the NAFLD fibrosis score were proven to be reliable in excluding advanced fibrosis in patients with NAFLD and are often used in daily practice to risk-stratify patients with NAFLD and T2DM[29].

All statistical analyses were performed using Matlab.

**RESULTS**

**Main characteristics**

Between January 2015 and April 2015, 44 patients (25 male, 19 female) were included based on the above mentioned criteria. The main characteristics of the patients are listed in Table 1. The mean age was 51,5 year ranging from 37 to 65. The average BMI was 32,2 kg/m². 41% have abnormal liver enzymes (elevation of either AST, ALT, AP or GGT). Total cholesterol ranged from 112 to 338 with an average of 181,5 mg/dl, TG from 69 to 311 with an average of 149,8 mg/dl. Fasting glucose varied between 73 and 353 mg/dl with a mean of 160,6 mg/dl. Mean HbA1c was 8%. 61% of the patients are on therapy with insulin. The numbers of criteria of MS according to the IDF ranged from 4 to 5, and all patients met the definition of MS. In our study group, 82% of the patients had NAFLD. 23%, 34%, 25% scored respectively 1, 2 and 3 on the USS whereas 18% had a USS of 0 and thus did not have NAFLD. The study group consisted of 57% men and 43% women. More men than women had NAFLD (84% men versus 78% women) and they also tend have a higher USS.

**Scores**

The results for the different scores are listed in Table 2. The percentage of patients that tested positive for the different scores for the Antwerp NASH score 1 and 2, the NASH activity score,
the Antwerp NAFLD significant fibrosis score and the Antwerp NAFLD advanced fibrosis score, the FIB-4 score and the NAFLD fibrosis score were respectively 16%, 84%, 14%, 11%, 14%, 9% and 50%. The percentage of patients who tested negative were respectively 27%, 0%, 50%, 82%, 57%, 23%, 0%. The remaining fall in between, so no conclusion can be made based on the scores.

From the 44 patients, 26 had normal liver enzymes (ALT, AST, AP, GGT). But from these 26, 12 scored positive on at least 2 scores and are likely to have fibrotic liver disease. The correlation between having normal liver enzymes and the total score (sum of all the scores) was negative (-0.3). Meaning that patients with elevated enzymes more often score higher and have more severe disease. From the 26 patients with normal liver enzymes, 23 had NAFLD according to liver US. Five patients with elevated liver enzymes had no abnormalities on liver US.

**Correlations**
The correlation between following insulin therapy for diabetes and the different scores was negative. This means that patients taking insulin for their diabetes on average have a better score (less chance to have the disease according to the score), then people following no insulin therapy. There was no significant correlation between the duration of diabetes and the different scores.

For patients treated with insulin, the correlation between C-peptide and HbA1c was strongly positive, meaning a higher C-peptide in general resulted in a higher HbA1c. However, for patients who were not treated with insulin, there was almost no correlation between C-peptide and HbA1c.

The correlation between the different scores was weakly positive (between 0.0633 and 0.2546).

**DISCUSSION**
NAFLD is called the disease of the century, with an enormous increase in prevalence in the Western world. In some patients, the natural history of NAFLD is benign, but in others there is progression to NASH and cirrhosis with all its complications. Based on clinical, biochemical and radiological data, it is impossible to predict the evolution in a certain patient. Up to now, liver biopsy remains the gold standard to identify patients at risk. Therefore, noninvasive tools are
urgently needed to differentiate between stages of severity in NAFLD. An evident solution is the use of a score that exists of noninvasive parameters that are easily obtained in daily practice. Many score have been developed to screen for fibrosis, and even for NASH, but none of these were validated in patients with T2DM, a population that is at a high risk of developing NAFLD. This study took a closer look at existing scores and applied them to T2DM patients with steatosis on ultrasound.

82% of the study group has NAFLD, which is slightly higher than the 74% reported in literature [11]. This number though is based on small study groups since studies on T2DM patients and NAFLD are still scarce.

Literature reports the presence of MS in 77 to 88% of the patients with T2DM [20]. In our study, every patient met the criteria of MS according to the IDF, this is due to the fact that patients in the UZ Brussels are under a strict therapy for dyslipidemia and blood pressure. This also means that most of them are overweight or obese. The mean BMI of 32,2 kg/m² confirms this, as does the mean waist of 108,6 cm.

In this study more than half of the patients (52%) had normal liver enzymes, but had NAFLD according to liver US. This confirms that patients do not necessarily have elevated liver enzymes when the liver has already been affected. According to different small studies in patients with diabetes and histologically proven NASH, elevated enzymes were present in less than 20% [20]. But, although ALT and AP can be within normal limits, they are significantly higher in patients with NASH. The same is true for the AST to ALT ratio in patients with fibrosis.

**The Antwerp NASH score 1**
The Antwerp NASH score 1 predicts the diagnosis of NASH according to the definition by Brunt et al. The parameters ALT>40, USS and fasting C-peptide are used to calculate the score. The mean, standard deviation and range of these parameters in our study is comparable with the previous study performed in obese people, although the average fasting C-peptide is slightly lower in our study, where all patients have diabetes. 27% of the patients fall under the first cutoff, suggesting they do not have NASH. This is very close to the 28% in the original study. Using the second cutoff, 16% of the patients in our study have NASH, which is 10% less than in the study group for the overweight patients. This could be explained by the lower C-peptide.
The Antwerp NASH score 2
The Antwerp NASH score 2 also predicts the diagnosis of NASH, but according to the definition by Kleiner et al instead of Brunt et al. To calculate this score, the ALT [U/L] value, the USS and the number of IDF criteria for MS are used. For the ALT and the USS, the same is true as for the Antwerp NASH score 1. However, the number of IDF criteria for MS is always four or five in our population, while in the group of obese people, the range was between one and five. The number of IDF criteria in patients with diabetes is higher, because whenever treatment is taken for one of the criteria, the score immediately goes up with one. People with diabetes, who are in treatment at UZ Brussel are under a strict regime for controlling dyslipidemia and hypertension and are often treated for it. It is likely that this factor is less relevant in diabetic patients who often take this treatment preventively.

The high score for the number of IDF criteria for MS results in a high Antwerp NASH score 2 for all the patients in our study. This results in 84% of our patients having an Antwerp NASH score above the second cutoff value, meaning they will most certainly have the diagnosis of NASH according to the definition of Kleiner et al. This percentage is much higher than for the Antwerp NASH score 1 (16%), and is probably false positive because of the above mentioned reasons.

The Antwerp NASH Activity Score
The Antwerp NASH Activity Score correlates with the severity of NASH as expressed by the NASH activity score (NAS), a score calculated by making the sum of scores for steatosis, lobular inflammation and ballooning. In this score, again ALT, USS and fasting fasting C-peptide are used, which are the same parameters used for the Antwerp NASH score 1. When the definition of NASH by a NAS \( \geq 5 \) is used, only 14% of the patients would have NASH, which is even lower than for the Antwerp NASH score 1. This percentage is in great contrast with the Antwerp NASH score 2, but correlates better with the results obtained in overweight patients.

The Antwerp NAFLD Significant and Advanced Fibrosis Score
The Antwerp NAFLD significant fibrosis score and the Antwerp NAFLD advanced fibrosis score predict respectively the presence of significant fibrosis and the presence of advanced fibrosis. The waist circumference, fasting C-peptide and AST are used. In our study group 11% have significant fibrosis and 14% have advanced fibrosis meaning that one quarter of patients had at least significant fibrosis. In the overweight group for which the score was developed,
19.5% had significant fibrosis and 7.3% advanced fibrosis, which is in the same range. The small differences might be explained by 2 parameters used in the formula: waist and AST. The average waist for our group is slightly smaller than the group used for designing the score for overweight or obese patients. The AST on the other hand, is much larger in the group of patients with diabetes.

**The FIB-4 and the NAFLD fibrosis score**

For comparing the former scores, two extra scores are used, namely the NAFLD fibrosis score and the Fibrosis 4 score (FIB-4). For all the scores evaluated, there are two cutoff values used, if the patient is below cutoff 1, he most certainly does not have the disease. If he scores above cutoff 2, he is very likely to have the disease. If he has a score in between the two cutoff values, a biopsy is necessary to make a conclusion. According to the FIB-4 score, only 9% of the patients have fibrosis, and according to the NAFLD fibrosis score 50%. This could be explained by the fact that the NAFLD fibrosis score takes into account hyperglycemia and BMI, unlike the FIB-4 score, which was developed for patients with viral hepatitis.

When comparing the different scores from the same patient, we observed that patients, who have a high value for one score, also tend to have a high value for the other scores, having a positive correlation between all the scores. Since they all indicate the same type of disease, this is an indication that the correlations that were made between certain parameters and having fibrosis for obese patients are valid as well for patients with diabetes. However, for the different scoring systems, distribution of groups under the first cutoff value, between the two cutoff values, and above the second cutoff value, are very different. From this, it can be concluded that, even in the formulas for the scoring systems will apply for patients with diabetes, the cutoff values will have to be adjusted. All these observations need to be verified by means of a liver biopsy. Besides that, it is also necessary to verify if other parameters, more specifically linked to diabetes should be included in the formula to obtain more accurate results.

The correlation between following therapy for diabetes and the different scores is negative. If this is indeed the case needs to be validated by performing biopsies. It is also possible that the therapy only suppresses some factors linked to the disease, and not the disease itself.

The values of the parameters ALT, AST, platelets and albumin are in the same range as they were for the tested cohort for which the score was designed. This could suggest that the scores
may be correct for patient with T2DM, but a correlation to patients with T2DM needs to be shown before this can be concluded. On the other hand, parameters not used in these scores, but important in T2DM that may have an influence on NAFLD, were not studied here, so no conclusions can be made.

The major shortage of this study is clear: no liver biopsies were preformed. After performing biopsies, a receiver operating characteristic curve (ROC-curve) can be drawn, and compared to the ROC-curve for the examined score in this study. If the results for the two groups are not similar, this could mean two things. One explanation could be that the formula to calculate the score is only valid for a specific range of the aforementioned parameters, but in this case this would rather seem odd because of the similar distribution of these parameters in both design cohorts. Another reason could be that one of the parameters is less or not relevant when the patient had T2DM. There could also be another important parameter, which is strongly linked to diabetes, which is missing in this formula, of which the correlation with the presence of NASH could not be shown when the design cohort is limited to obese people.

This means that no conclusion can be drawn. Further studies are required to validate existing scores, or create new scores for patients with T2DM.

**CONCLUSION**

The aim of this study was to get a better perspective on NAFLD in patients with T2DM. This was done by collecting different standard parameters of these patients and by implementing existing scores that screen for NAFLD. We found that 82% of our patients with T2DM have steatosis on ultrasound and that patients with NAFLD often do not have elevated liver enzymes, which is also reported in literature. This implicates that liver enzymes alone are not suited to screen for NAFLD, emphasasing the need to develop a simple, non-invasive method to screen for the different stages of NAFLD. According to our results, based on different scores, 9 to 50% of the patients have fibrosis. Even if existing scoring systems were not validated for this specific population, we found a good correlation between different scores and we believe that at least some of the scores have the potential to screen for NASH in T2DM patients. The present study should be considered as a pre-study, but the results that we have obtained should increase awareness of the magnitude of significant liver disease in T2DM patients. The next step in our
research will be performing liver biopsies in T2DM patients in whom steatosis is present on liver ultrasound and correlate the biopsy result with the different scores. If the scores cannot be validated in our population, we will develop a new specific score for T2DM patients.

REFERENCES


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Table 1. Main Characteristics of het Overall Patient cohort (n=44).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase BMI, body mass index; GGT, gamma glutamyl transpeptidase; HBA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; IDF, International Diabetes Federation; LDH, lactate dehydrogenase; SD, standard deviation; TG, triglycerides; USS, ultrasound steatosis score.
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<th>NASH2</th>
<th>NAS</th>
<th>NAFLD1</th>
<th>NAFLD2</th>
<th>NAFLD fibrosis</th>
<th>FIB-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>-0.166 ± 3.07</td>
<td>2.95 ± 5.73</td>
<td>3.05 ± 4.36</td>
<td>-2.46 ± 7.71</td>
<td>-3.34 ± 23.7</td>
<td>-0.69 ± 1.9</td>
<td>1.7 ± 8.88</td>
</tr>
<tr>
<td>Range</td>
<td>-3.2461</td>
<td>-0.848</td>
<td>-0.0807</td>
<td>-5.055</td>
<td>-6.79</td>
<td>-32686</td>
<td>0.2966 20.3264</td>
</tr>
<tr>
<td>Percentage</td>
<td>27.3/56.8/15.9</td>
<td>0/15.9/84.1</td>
<td>50/36.4/13.6</td>
<td>81.8/6.8/11.4</td>
<td>56.8/29.6/13.6</td>
<td>22.7/27.3/5.0</td>
<td>0/90.9/9.1</td>
</tr>
</tbody>
</table>

Table 2. Results for the Tested Scores

NASH 1 and 2, the Antwerp NASH score 1 and 2; NAS, the Antwerp NASH severity score; NAFLD 1 and 2, the Antwerp NAFLD significant fibrosis score and the Antwerp NAFLD advanced fibrosis score; NAFLD fibrosis, the NAFLD fibrosis score; FIB-4, the fibrosis 4 score; SD, standard deviation.