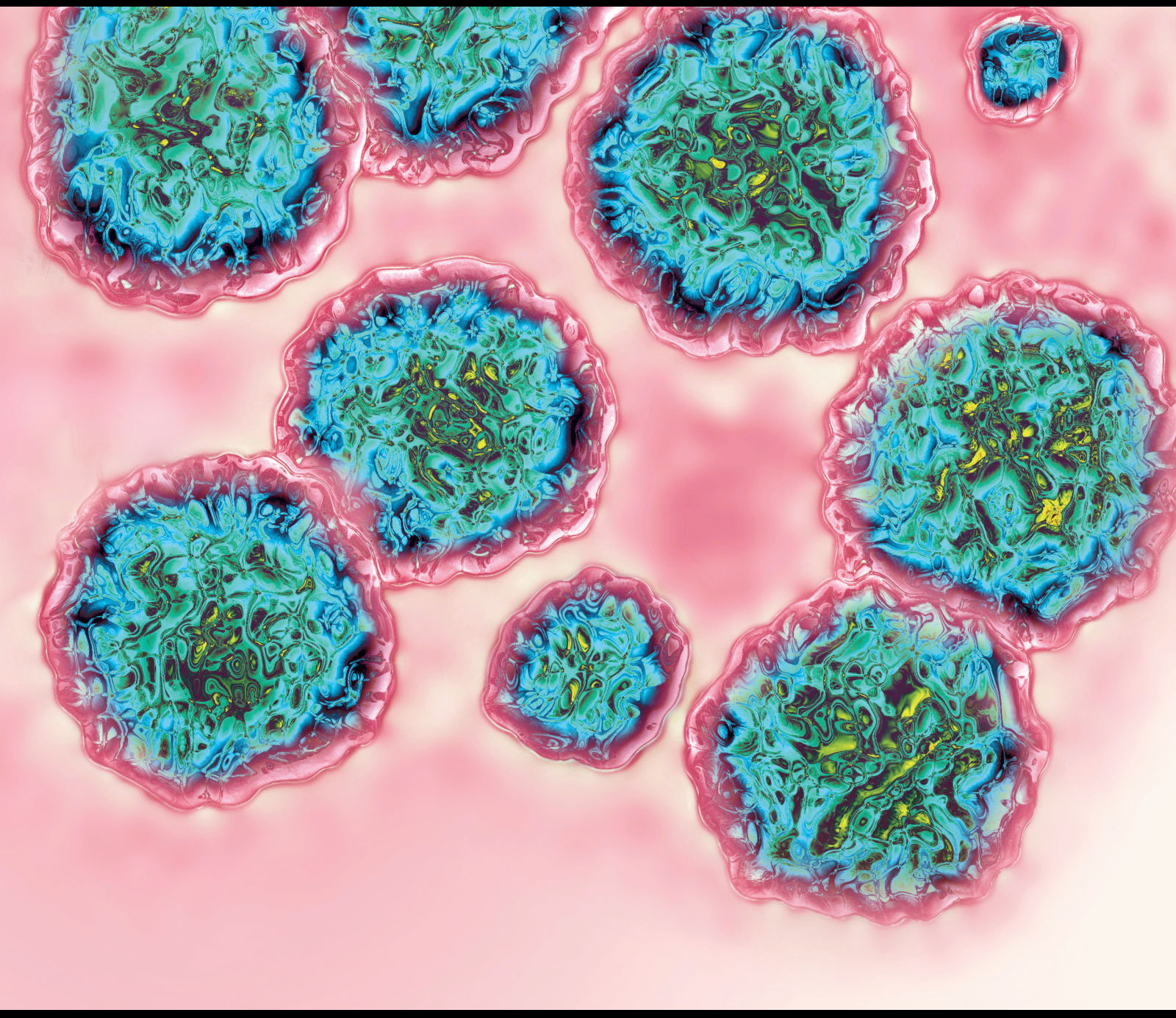
Canadian Journal of Gastroenterology and Hepatology

Non-Alcoholic Fatty Liver Disease and Extra-Hepatic Manifestations

Lead Guest Editor: Branka Filipović
Guest Editors: Dan Dumitrascu and Goran Hauser



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Research Article

Anatomical Brain Changes and Cognitive Abilities in Patients with Obstructive Sleep Apnea Syndrome and Nonalcoholic Fatty Liver Disease

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Obstructive sleep apnea (OSA) is characterized by repetitive complete or partial collapse of the upper airway and reduction of airflow during sleep. It is associated with significantly increased daytime muscle sympathetic nerve activity thought to result from the repetitive intermittent periods of hypoxemia during sleep and brain alterations that are likely to result. Different brain regions are affected by subsequent hypoxia/anoxia. Neurodegenerative processes result in measurable atrophy of cortical gray matter in the temporal lobes and posterior cingulate cortex, as well as in subcortical structures such as the hippocampus, amygdala, and thalamus. This study involved a group of firstly diagnosed, therapy-naive, nonalcoholic fatty liver disease (NAFLD) patients, out of which 144 (96 males and 48 females), aged 34–57 (mean 47.88 ± 6.07), satisfied the recruiting criteria for the study and control groups. All the patients underwent MRI scanning, polysomnography testing, and cognitive evaluation. Cognitively, worse results were obtained in the group with OSA ($p < 0.05$) and NAFLD ($p = 0.047$). A significant decrease in volumes of cortical and subcortical structures was revealed ($p < 0.001$). In conclusion, brain deterioration followed by cognitive impairment is, most likely, the result of intermittent hypoxia and anoxia episodes that initiate the domino process of deteriorating biochemical reactions in the brain.

1. Introduction

Obstructive sleep apnea (OSA) is characterized by repetitive complete or partial collapse of the upper airway and reduction of airflow during sleep. It is associated with significantly increased daytime muscle sympathetic nerve activity thought to result from the repetitive intermittent periods of hypoxemia during sleep and brain alterations that are likely to result [1, 2]. Some studies based on functional magnetic resonance imaging revealed attenuated signals in numerous regions of the brain: in the prefrontal, cingulate, and precuneus cortices; in the retrosplenial cortex, caudate nucleus, hippocampus, and parahippocampal regions; and within the dorsolateral pons, rostral ventrolateral medulla, medullary raphe, and midbrain [3, 4].

Studies conducted in younger and middle-aged adults indicate that the effects of sleep fragmentation and nocturnal hypoxemia probably support the cognitive deficits associated with OSA [5]. Sleep disruption with the compounding effect of hypoxemia could have deteriorating effects on brain

integrity and morphology [6]. A wide range of cerebral gray matter changes has been associated with OSA, including cortical or volume changes across the temporal lobe and prefrontal cortex, and subcortical structures involving the hippocampus, thalamus, and cerebellum [7, 8].

Nonetheless, a relative paucity of investigations dealing with the interrelationships between OSA, brain integrity, and cognitive decline in older adults should be noted. As adults age, they may experience neurodegenerative processes resulting in measurable atrophy of cortical gray matter in the temporal lobes and posterior cingulate cortex, as well as subcortical structures such as the hippocampus, amygdala, and thalamus. These changes are evident even in the transitional or "at-risk" stages between normal aging and dementia, defined as those with subjective memory concerns and mild cognitive impairment [9–12].

A study by Elliott et al. [13] examined the cognitive symptoms of nonalcoholic fatty liver disease (NAFLD). They observed that increased cognitive difficulties were associated independently with functional difficulties in the NAFLD group compared with a healthy control group. A large cross-sectional study performed by Seo et al. [14] examined the association between NAFLD and cognitive impairment using computer-based tests of attention and reaction times.

They estimated that NAFLD was associated independently with reduced cognitive performance independent of cardiovascular disease and its risk factors. Previous studies on animals have shown a very strong connection between OSA and NAFLD [15]. Mesarwi et al. [15] showed that animals exposed to intermittent hypoxia (IH) have elevated blood pressure and develop sympathetic overactivation, atherosclerosis, and glucose as well as lipid dysregulation. OSA causes IH by recurrent collapse of the upper airway during sleep, as measured by peripheral oxyhemoglobin saturation. It may seem intuitive that arterial desaturation would result in intermittent tissue hypoxia as well.

However, no study has examined the tissue-specific effects of recurrent airway closure in humans. A few studies have shown that liver enzymes may be acutely elevated in OSA and are lowered with CPAP [16], and at least one study has shown that serum creatine phosphokinase similarly may be elevated in OSA and reduced by CPAP [17].

We aimed to assess whether OSA is associated with structural brain changes in various brain regions and whether OSA consecutively leads to cognitive impairment compared to NAFLD patients. Our goal was to compare the differences in cognitive functions in individuals with OSA and NAFLD relative to those with NAFLD but without OSA. We hypothesized that significant differences in cognitive statuses exist between those two groups of interest.

1.1. Materials and Methodology. This study involved a group of firstly diagnosed, therapy-naive NAFLD patients, out of which 144 (96 males and 48 females), aged 34–57 (mean 47.88 ± 6.07), satisfied recruiting criteria for the study and control groups. The grouping criterion for the division into the studied and control group was the presence of the OSA, so the studied group included the patients with NAFLD and OSA, and the controls were the individuals with NAFLD but without OSA. The study was approved by the Ethical Committee of the Clinical and Hospital Center "Dr Dragisa Misovic–Dedinje," Belgrade.

All the participants were acquainted in detail with the study aim and design before entering the program. They all signed a written consent afterward.

A selection flow diagram is shown in Figure 1.

Recruiting criteria were as follows:

(1) Older than 18.

(2) No previous history of viral hepatitis of any kind, haemochromatosis, autoimmune hepatitis, cirrhosis, or other chronic liver diseases.

(3) No presence of severe cardiopulmonary disease.

(4) The absence of endocrinological disorders: hypothyroidism, hypercorticism, and syndrome of the polycystic ovaries.

(5) No history or clinical signs of excessive alcohol abuse (>20 g/day for males and >10 g/day for females).

(6) No neuropsychiatric disease involving signs of any kind of dementia, and/or neuropsychiatric medication history, or any other hepatotoxic drugs.

(7) No visible traces of illicit drugs abuse. Negative urine multiple drug test on 10 kinds of drugs: cannabinoids, opiates, amphetamines, 3, 4-methylenedioxymethamphetamine, cocaine/crack, benzodiazepines, tricyclic antidepressants, barbiturates, methadone, and buprenorphine.

(8) No visible focal or diffuse changes in the gray matter of the brain on MRI.

(9) Fazekas score 0 on MRI scan. Fazekas score is the estimated level of the white matter vascular changes and is the aftermath of the brain vessels' atherosclerotic changes.

(10) Absence of any rheumatologic disease.

(11) Patients who used antidiabetic drugs, insulin, antilipemic drugs, uricosuric drugs, steroids, and oral contraceptives were excluded from the study.

2. Volumetric Procedures

Volume measurements of the gray and white matter and lateral ventricles of the brain were performed on 3D T1-weighted MR images (Phillips Inc. Holland). Acquisition parameters were as follows: TR = 9.8 ms; TE = 4.6 ms; flip angle = 8; section thickness = 1.2 mm; number of sections = 120; no section gap; whole-brain coverage; FOV = 224 mm; matrix = 192; reconstruction matrix = 256. Routine T2-weighted and FLAIR images were performed to rule out a mass lesion as a contributory factor to memory loss or cognitive decline. The structures were manually outlined and compared with automatic extraction of the regions of interest in commercially available software. The software finally computed the volumes required.

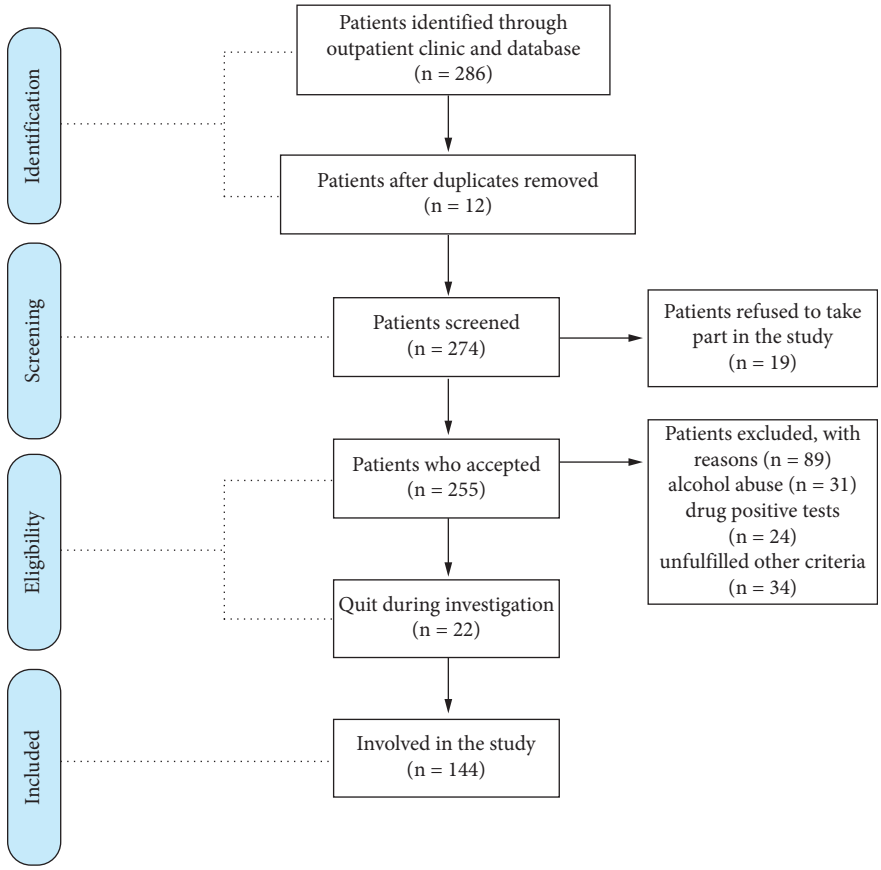

Figure 1: Patient selection flowchart.

2.1. Cognitive Testing. After the diagnostic procedures, all the subjects underwent psychological testing of cognitive impairments using the Montreal Cognitive Assessment (MoCA) test, Serbian version. The test has several levels of testing: alternating connection (connect Figure 1 with letter A, then A to 2, to B, etc.), visuoconstructive abilities (draw a cube and a clock in 11:10 position of clock hands), memory (repeating numbers in the same and reverse order), attention (tap whenever you hear a letter A), serial subtraction of 7, starting with hundred, $100 - 7 = 93$, $93 - 7 = 86$, etc.), sentence repeating, and verbal fluency. The maximal score is 30, 26 being the threshold for normal cognitive functioning [18].

2.2. Laboratory Analysis. For body weight and height, the patients were measured in bare feet and light clothing in the morning with the same equipment. Body mass index (BMI) was calculated by dividing body weight by height square (kg/m^2).

Fasting blood was taken in the morning for the measurement of serum glucose, and lipid profile comprising total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and triglycerides (TG). Adipokines, adiponectin, and leptin were analyzed and compared. All the tests were run by AO-BK-200 mini Auto Biochemistry Analyzer, Alpha Omega Electronics, Madrid, Spain.

2.3. Polysomnography. All participants, examined and controls, underwent polysomnography (PSG) in the sleep department. It was performed within four weeks of the MRI scan and neuropsychological testing. Nocturnal PSGs were collected on an ambulatory recording system with the Alice PDx portable diagnostic recording device (Philips Respironics), together with nasal airflow which was recorded with the nasal pressure transducer. Respiratory effort was assessed using thoracic and abdominal bands; blood oxygen saturation was revealed by pulse oximetry. Patients were advised not to disturb their usual bedtime weekly rhythm and were required to abstain from caffeinated beverages (coffee, caffeinated soda) at least eight hours before and, especially, during PSG data collection. The study was reported by an accredited sleep physician.

Sleep staging was scored according to the criteria of the American Academy of Sleep Medicine [19]. Apnea was defined as decrements in airflow ≥90% from baseline for ≥10 s. Hypopnea was defined as a 30% or greater decrease in flow lasting for ≥10 s and was associated with a 4% or greater oxyhemoglobin desaturation. The numbers of apneas and hypopneas per hour of sleep were calculated to obtain the apnea-hypopnea index (AHI). The oxygen desaturation index (ODI) was defined as the number of dips in oxygen saturation (SpO_2) ≥4% per hour of total sleep time. OSA was defined as normal: $AHI < 5$; mild sleep apnea: $5 \leq AHI < 15$;

moderate sleep apnea: $15 \leq \text{AHI} < 30$; and severe sleep apnea: $\text{AHI} \geq 30$ events/h.

The atherogenic index of plasma (AIP) represents the risk for atherosclerosis. It is calculated as the logarithmic value of the triglyceride/HDL score. The risk was interpreted as follows: AIP < 0.11 low risk; AIP = 0.11–0.21 intermediate risk; and API > 0.21 increased risk. The following index values were analyzed: cholesterol/HDL, triglycerides/HDL, and HDL/LDL.

2.4. Ultrasonography Evaluation. The liver was assessed as normal when the consistency was homogeneous, displayed fine level echoes, minimally hyperechoic or even isoechoic in contrast to the regular renal cortex. Mild steatosis was evaluated as a minor increase in liver echogenicity. In moderate steatosis, there were visual images associated with intrahepatic vessels, the slightly damaged diaphragm, and the existence of increased liver organ echogenicity. Severe steatosis was evaluated as a marked increase in hepatic echogenicity, poor penetration of posterior segment from the right lobe of the liver, and poor or no visual images from the hepatic vessels and the diaphragm [20].

FibroScan® (Echosens group) was used to determine the fibrosis grade in the liver parenchyma. The normal range for a FibroScan is between 2 and 7 kPa. The average normal result is 5.3 kPa. The results vary based on the liver disease in question. For NASH/NAFLD there are 4 stages of scarring: • F_0 to F_1 means no scarring or mild fibrosis, 2–7 kPa; • F_2 is moderate fibrosis, 7–10 kPa; • F_3 is severe fibrosis, 10–14 kPa; and • F_4 is cirrhosis or advanced fibrosis higher than 14 kPa.

2.5. Statistical Evaluation. Statistical testing was performed by the commercially available software (SPSS 17.0, Inc., Chicago Il, US). Besides measures of the central tendency (mean and standard deviation (SD), minimum and maximum), potential differences of mean values were assessed with one-way analysis of variance (ANOVA) with the Bonferroni post hoc correction, Student's t-test for independent samples for parametric, and the chi-squared test for nonparametric data. The correlation between the variables was estimated using Spearman and Pearson's correlation coefficient. Multivariate linear regression was performed with volumes of brain structures as a dependent variable, while OSA and NAFLD degrees were independent predictors, adjusting for gender, age, BMI, cholesterol level, adiponectin, and leptin. Statistical hypotheses were analyzed at the level of significance of 0.05.

3. Results

3.1. Demographic Data. Demographic data are shown in Table 1. Males dominated in the groups with sleep apnea. The body mass index was significantly higher among the persons with OSA.

3.2. Laboratory Results. The concentration of serum triglyceride, HDL, and LDL differed significantly: patients with severe OSA had the lowest concentration of HDL and the highest level of LDL. All the examinees with OSA were at very high risk for atherosclerosis (all above 0.51 risk index).

3.3. Brain Volumes Changes. Although total brain volumes among the groups observed did not differ significantly, volumes of the structures of interest were significantly lower in the group of examinees with OSA. Higher volumes were obtained for the lateral ventricles on both hemispheres in OSA suffering patients, while volumes of the amygdaloid complexes did not differ significantly (Table 2).

3.4. Association between Liver Steatosis and Fibrosis and OSA. The severity of OSA differed among the observed groups with liver steatosis (Table 3). The patients with serious steatosis were numerous among those with severe OSA. The severity of NAFLD is associated with the increase in OSA severity (rho = 0.214; $p = 0.010$). Level of fibrosis estimated with the Fibro scan correlated with the grade of steatosis ($B = 0.56$, beta = 0.73, $t = 13.09$, significance $p < 0.0001$), as well as with the severity of OSA ($B = 0.89$, beta = 0.13, $t = 2.37$, significance $p = 0.019$).

3.5. Level of Cognitive Deficit. According to the MoCA score, the groups divided by the grade of liver steatosis differed ($F = 2.72$, DF = 3, 140, $p = 0.047$).

Regarding the severity of OSA, the level of the cognitive deficit did not differ among the obtained groups.

Discriminative function analysis outlined cognitive level as the only parameter of importance for the classification of a newly obtained patient into one of the groups of interest: equation = $-9.19 + 0.37 \times \text{MoCA}$; centroids for groups: mild, −0.17; moderate, −0.63; severe, 0.25 and cutoff points: mild to moderate, −0.23; moderate to severe, −0.195; goodness of classification, 71.50%.

3.6. Polysomnography Results. Polysomnographic parameters had an inverse influence on the volumes of the structures of interest. The tested subjects had lower volumes when both AHI and ODI were higher (Table 4). Atherogenic index of plasma correlated with AHI ($r_2 = 0.48$, constant = 17.63, $b_1 = 12.27$, $p = 0.015$), ODI ($r_2 = 0.75$ constant = 16.182, $b_1 = 14.29$ $p = 0.02$), and BMI ($r_2 = 0.106$, constant = 28.17, $b_1 = 5.45$, $p < 0.001$).

3.7. Brain Volumes in Patients with OSA. In the multivariate regression analyses the patients with higher levels OSA showed a significant reduction in all volumes of brain structures except for amygdaloid complex and white matter volume (Table 5).

4. Discussion

The study aimed to reveal whether OSA is associated with structural brain changes in diverse brain regions and whether the grade of liver steatosis influences both OSA

Table 1: Demographic parameters of examined population.

Group according to sleep apnea severity	Examined ($N = 68$)	Controls ($N = 76$)	Total ($N = 144$)	Significance
Parameter				
Age (years ± SD)	47.88 ± 6.07	47.62 ± 6.97	46.94 ± 9.00	NS
Gender (male/female)	58/10	38/38	96/48	$p < 0.001$
Education level (grammar/high school/university)	3/7/12	25/33/18	43/70/31	NS
Body mass index (BMI, kg/m^2)	35.34 ± 7.31	34.95 ± 8.27	31.90 ± 6.61	$F = 807.33$, DF = 3.140, $p < 0.001$
Leptin (ng/mL) m/f	11.36 ± 1.97 22.78 ± 3.28	4.39 ± 2.17 11.44 ± 3.23	10.39 ± 2.53 14.55 ± 3.11	$F = 5.66$, DF = 3.140, $p < 0.001$
Adiponectin (ng/mL) m/f	8.06 ± 0.97 11.14 ± 2.03	8.8 ± 1.44 13.71 ± 3.63	8.19 ± 2.44 11.43 ± 3.69	NS
Glucose (mmol/l)	5.31 ± 0.65	5.75 ± 1.55	5.52 ± 1.28	NS
C reactive protein (mg/l, mean ± SE)	3.86 ± 1.27	3.74 ± 0.39	3.39 ± 0.25	NS
Cholesterol (mmol/l, mean ± SD)	5.19 ± 0.63	5.74 ± 1.18	5.57 ± 1.11	NS
HDL (mmol/l, mean ± SE)	0.95 ± 0.04	1.37 ± 0.07	1.18 ± 0.04	$F = 8.94$, DF = 3.140, $p < 0.001$
LDL (mmol/l, mean ± SE)	3.23 ± 0.17	3.27 ± 0.12	3.44 ± 0.08	$F = 2.76$, DF = 3.140, $p < 0.05$
Triglycerides (mmol/l, mean ± SE)	2.68 ± 0.35	1.86 ± 0.13	2.24 ± 0.12	$F = 7.64$, DF = 3.140, $p = 0.009$
Cholesterol/HDL ratio (mean ± SE)	6.02 ± 0.33	4.64 ± 0.18	5.27 ± 0.18	$F = 8.035$, DF = 3.140, $p < 0.001$
Triglycerides/HDL ratio	3.32 ± 0.48	1.62 ± 0.15	2.41 ± 0.19	$F = 7.71$, DF = 3.140, $p < 0.0001$
AIP	0.52	0.21	0.38	$F = 8.01$, DF = 3.140, $p < 0.001$
HDL/LDL ratio (mean ± SE)	0.32 ± 0.03	0.43 ± 0.04	0.38 ± 0.02	NS
AHI per hour (mean ± SE)	12.23 ± 5.49	2.95 ± 0.12	12.16 ± 1.31	$F = 29.44$, DF = 3>140, $p < 0.001$
ODI per hour (mean ± SE)	14.35 ± 4.51	2.78 ± 0.13	11.08 ± 1.24	$F = 25.64$, DF = 3.140, $p < 0.001$
Total brain volume (cm^3, mean ± SE)	1441.19 ± 32.31	1403.37 ± 18.85	1398.96 ± 13.16	NS
MoCA score (mean ± SD)	24.23 ± 3.14	25.53 ± 3.20	25.28 ± 3.04	$F = 2.72$, DF = 3.140, $p < 0.05$

NS: not significant.

appearance and subsequent cognitive alterations, as the result of IH.

Our results indicate that the volume changes of the overall cortex and basal nuclei are related to AHI and ODI as the main parameters and both strongly influenced volume decrease. These results are in correlation with previous studies which showed changes in volume values of the brain structures of interest. Kim et al. [21] investigated the effect of long-term treatment on brain volume in patients with OSA and their results have shown a significant increase in volume in the medial prefrontal cortex, superior frontal cortex, precuneus, and posterior temporal cortex, as well as in the dentate gyrus of hippocampus, thalamus, and cerebellum including the dentate nucleus. Fatouleh et al. [3] conducted a study on patients with OSA and their results showed significant changes in volume in the left and right parts of the insula, dorsolateral prefrontal cortex, dorsal precuneus, sensorimotor cortex, and posterior temporal cortex, as well as anterior cingulate cortex, retrosplenial cortex, and caudate nucleus. All mentioned brain structures, previously hippocampus and medial prefrontal cortex, are involved in the regulation of sleep and have direct anatomical connections with the pre-Bötzinger Complex (preBötC), a compact medullary region essential for generating normal breathing rhythm and pattern [22].

OSA causes nocturnal intermittent hypoxemia and sleep fragmentation in response to oxygen desaturation. Some investigators indicated that OSA, vascular depression, and cognitive impairment are linked to several pathologic processes in the cerebral microvascular and neurovascular systems [23, 24]. In OSA repetitive episodes of the intracranial blood flow, an unexpected increase during apneic episodes caused damage to the endothelial cells of small arteries and arterioles, which result in decreased endothelial vasodilator production such as nitric oxide. Moreover, IH during sleep in patients with OSA can contribute to apoptosis and atrophy within the hippocampal structure, resulting in learning, mnemonic, attentional, and executive function deficits [24]. Filipovic et al. [25] investigated the possible correlation between cognitive status and NAFLD using the MoCA test, finding a lower MoCA score and a reduction in white and gray brain volumes in NAFLD patients. The patients with NAFLD have a risk four times higher than manifesting lower cognitive abilities and depleted cognitive performance and deficit.

The major risk factors for OSA include obesity, male sex, alcohol and smoking habits, a family history of OSA, and upper airway structural abnormalities such as a large neck girth and craniofacial abnormalities. NAFLD is most commonly associated with metabolic risk factors, such as obesity, diabetes mellitus type 2, and elevated triglyceride levels, but some recent studies have reported that chronic intermittent hypoxia (CIH) can be an independent risk factor to induce liver damage [26, 27]. OSA and episodes of repetitive IH induce insulin resistance and dyslipidemia which are involved in NAFLD pathogenesis. CIH increases the expression of the hypoxia-inducible factor 1-alpha and that of downstream genes involved in lipogenesis, increasing β-oxidation and, consequently, leading to exacerbation of oxidative stress in the liver. OSA also disrupts the gut-liver axis, increasing intestinal permeability with a possible role of gut microbiota in the link between OSA and NAFLD [28].

Table 2: Brain volumes according to the sleep apnea of the observed groups.

Group according to sleep apnea severity	Mild ($N = 11$)		Moderate ($N = 14$)		Severe ($N = 43$)		Controls ($N = 76$)		Total ($N = 144$)		Significance	
	L	R	L	R	L	R	L	R	L	R	L	R
Caudate nucleus volume (cm^3, mean ± SD, L/R)	3.76 ± 0.11	3.80 ± 0.15	3.77 ± 0.04	3.81 ± 0.16	3.79 ± 0.15	3.85 ± 0.14	4.5 ± 0.65	4.56 ± 0.64	4.22 ± 0.6	4.17 ± 0.6	F 3.140 = 26.33, $p < 0.001$	F 3.140 = 27.93, $p < 0.001$
Putamen volume (cm^3, mean ± SD, L/R)	5.94 ± 0.10	5.95 ± 0.10	5.97 ± 0.11	5.98 ± 0.11	5.97 ± 0.12	5.99 ± 0.12	6.31 ± 0.62	6.30 ± 0.70	6.15 ± 0.49	6.16 ± 0.54	F 3.140 = 6.51, $p < 0.001$	F 3.140 = 5.03, $p = 0.002$
Globus pallidus volume (cm^3, mean ± SD, L/R)	2.13 ± 0.10	2.12 ± 007	2.13 ± 0.08	2.61 ± 0.46	2.18 ± 0.79	2.12 ± 0.07	2.61 ± 0.46	2.58 ± 0.40	2.38 ± 0.41	2.36 ± 0.37	F 3.140 = 24.20, $p < 0.001$	F 3.140 = 27.06, $p < 0.001$
Thalamus volume (cm^3, mean ± SD, L/R)	6.29 ± 0.10	6.28 ± 0.09	6.27 ± 0.09	6.26 ± 0.09	6.28 ± 0.07	6.28 ± 0.07	7.46 ± 0.77	7.48 ± 0.90	6.90 ± 0.81	6.91 ± 0.79	F 3.140 = 52.12, $p < 0.001$	F 3.140 = 38.77, $p < 0.001$
Hippocampal formation volume (cm^3, mean ± SD, L/R)	2.82 ± 0.12	2.81 ± 0.12	2.77 ± 0.13	2.77 ± 0.13	2.80 ± 0.13	2.80 ± 0.13	3.99 ± 0.63	3.96 ± 0.68	3.43 ± 0.75	3.41 ± 0.77	F 3.140 = 78.02, $p < 0.001$	F 3.140 = 63.94, $p < 0.001$
Lateral ventricle volume (cm^3, mean ± SD, L/R)	7.48 ± 0.14	7.49 ± 0.14	7.49 ± 0.13	7.51 ± 0.13	7.50 ± 0.14	7.51 ± 0.14	5.86 ± 0.55	5.81 ± 0.54	6.63 ± 0.92	6.61 ± 0.94	F 3.140 = 183.72, $p < 0.001$	F 3.140 = 205.44, $p < 0.001$
Amygdaloid complex volume (cm^3, mean ± SD, L/R)	1.65 ± 0.11	1.64 ± 0.12	1.61 ± 0.08	1.61 ± 0.08	1.67 ± 0.08	1.68 ± 0.08	1.68 ± 0.19	1.69 ± 0.21	1.67 ± 0.15	1.68 ± 0.17	NS	NS
Prefrontal cortex volume (cm^3, mean ± SD, L/R)	140.45 ± 6.20	141.54 ± 6.07	143.43 ± 8.16	144.50 ± 8.05	145.93 ± 8.43	146.98 ± 8.36	161.16 ± 18.85	158.84 ± 19.32	153.30 ± 16.95	152.58 ± 16.46	F 3.140 = 15.41, $p < 0.001$	F 3.140 = 9.55, $p < 0.001$
Gray matter volume (cm^3, mean ± SD)	351.91 ± 4.91		348.79 ± 6.02		348.93 ± 7.57		341.43 ± 40.03		335.79 ± 29.93		F 3.140 = 35.34, $p < 0.001$	

Table 3: Distribution of steatosis severity among groups with different levels of OSA.

OSA	Grade of liver steatosis			
	Mild	Moderate	Severe	Total
Mild	11	0	0	11
Moderate	2	8	4	14
Severe	12	13	18	43
Without	36	25	15	76
Total	61	46	37	144

rho = 0.214; $p = 0.010$.

Several studies examined OSA influence on structural changes using magnetic resonance spectroscopy, the decreased N-acetyl aspartate, and choline concentration in prefrontal subcortical white matter. Alterations revealed the early structural changes such as neuronal loss and axonal damage [29–31].

In some magnetic resonance spectroscopy-guided studies, the left hippocampus was stressed as a region especially sensitive to intermittent hypoxia in OSA suffering patients [32]. Our results oppose the findings from the study of Yaouhi et al. [8] who reported a large variety of structures susceptible to OSA: bilateral inferior gyri, right temporal cortex, occipital cortex, right thalamus, left caudate nucleus and left globus pallidus, right hippocampal gyrus, cerebellar hemisphere on the same side, and vermis. Morrell et al. [33] reported changes in the left temporal lobe and right cerebellar hemisphere. Joo et al. [34] also pointed out a significant volume decrease in the left straight gyrus anterior cingulate cortex, the right insular cortex, caudate nucleus, amygdala–hippocampus complex, the inferior temporal gyrus, and cerebellum, in OSA patients versus healthy controls. The study performed by Torelli et al. [35] indicated the changes in bilateral caudate nucleus volume, but the change was relative and dependent on the presence of hypertension and cigarette smoking. If those two factors were checked for analysis, there would be no difference between the tested and control group. Cholesterol concentration differences were insignificant in our study, although the cholesterol/HDL ratio significantly differed between OSA patients and the controls. HDL concentrations showed higher values in the control group, while LDL was significantly higher in the group with severe OSA.

The atherogenic index of plasma was significantly lower in the controls but correlated with AHI, ODI, and BMI indexes. On the contrary, Sparks et al. [36] claimed that cholesterol concentrations may influence poorer cognitive performance. This disproportion may be related to the usage of different cognitive tests: in our study, the MoCA test was used as the main testing questionnaire, but Sparks and his team applied Mini-Mental State Examination for the evaluation of the cognitive state. According to the opinion of Rademeyer et al. [37], MoCA is a more potential screening tool for cognitive impairment. In the meta-analysis provided by Siqueira et al. [38], thirty-seven studies suggested that MoCA is a more sensitive tool for neurocognitive disorders detection because it assesses executive function and visuospatial abilities. Finally, the discriminative analysis function, according to our results, outlined the MoCA score as the only parameter of importance for the classification of a newly obtained subject into one of the groups of interest, selected by the OSA severity, with almost 80% of accuracy.

An et al. [39] stated that the higher intake of cholesterol negatively influences the cognitive state of the middle age Chinese population. Generally, lower values of HDL are indicated for the poorer cognitive state, or inversely, higher concentrations of HDL are indicators of cognitive improvement. The atherogenic index of plasma is nowadays considered a novel predictor of NAFLD, and NAFLD itself negatively influences the cognitive status as previously reported [40].

The role of two adipokines, leptin and adiponectin, in cognitive regulation is at least equivocal and insufficiently elucidated, considering literature data. Our patients with OSA had lower concentrations of adiponectin, but this difference was not statistically verified. It has already been published that adiponectin is lower in the examinees with OSA [41, 42], and OSA cohabitates with depression symptoms [43]. The patients with NAFLD are prone to depression [36]; furthermore, the depression appearance is related to the white matter loss which corresponds to the liver fibrosis grade [44].

Leptin values in our study are higher in patients with OSA and NAFLD, compared to the controls with NAFLD only. The highest values were noted in persons with severe OSA. Leptin is a potent ventilation stimulant acting on central respiratory control nuclei. The central satiety effects of leptin are abrogated in obesity. Leptin resistance is defined as a failure of high-circulating levels of leptin to decrease hunger and promote energy expenditure.

OSA and IH, powerful triggers of oxidative stress, increase peripheral leptin levels and also induce leptin resistance (for a detailed review, see [45]).

The statistical trend for lower leptin levels to be associated with higher cognitive scores was revealed in a large sample of 2731 subjects measured by MoCA. Excessive leptin per unit of fat was associated with lower total MoCA score and memory in black men and with higher MoCA scores in white men [46]. In our investigation, there was no possibility to explore different races because our examinees were strictly Caucasians. Whether this finding indicates that leptin has a different role in various anthropological types remains yet to be examined.

In our study, two indexes, characteristic for OSA, AHI, and ODI, were found to have a strong influence on the reduction of the volumes of almost all regions of interest. Only amygdaloid complexes were spared from the volume reduction. Total brain volume was found insignificantly different in our sample, which could be the result of larger lateral ventricle volumes and, subsequently, possible higher quantities of cerebrospinal liquor. OSA, that is, impaired AHI and ODI indexes, likely causes cognitive impairment through IH, hormonal imbalance, and/or systemic inflammation, either independently or via the resultant endothelial dysfunction that occurs. Still, the cognitive defect is only partially reparable after CPAP treatment (for a detailed review, see [47]). In most of the studies obtained, the

Table 4: Correlation matrix between polysomnographic parameters and volumes obtained.

Parameter	AHI per hour		ODI per hour		Significance AHI		Significance ODI	
	L	R	L	R	L	R	L	R
Caudate nucleus volume (cm^3)	$B = -0.015$ Const = 4.35 Beta = −0.37	$B = -0.015$ Const = 4.40 Beta = −0.39	$B = -0.015$ Const = 4.34 Beta = −0.38	$B = -0.015$ Const = 4.38 Beta = −0.38	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Putamen volume (cm^3)	$B = -0.01$ Const = 6.24 Beta = −0.25	$B = -0.01$ Const = 6.25 Beta = −0.22	$B = -0.07$ Const = 6.23 Beta = −0.22	$B = -0.07$ Const = 6.24 Beta = −0.198	$p < 0.005$	$p < 0.01$	$p < 0.01$	$p < 0.02$
Globus pallidus volume (cm^3)	$B = -0.01$ Const = 2.47 Beta = −0.38	$B = -0.01$ Const = 2.45 Beta = −0.36	$B = -0.01$ Const = 2.49 Beta = −0.35	$B = -0.09$ Const = 2.46 Beta = −0.37	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Thalamus volume (cm^3)	$B = -0.02$ Const = 7.19 Beta = −0.45	$B = -0.02$ Const = 7.20 Beta = −0.42	$B = -0.024$ Const = 7.16 Beta = −0.43	$B = -0.024$ Const = 7.18 Beta = −0.40	$p < 0.001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Hippocampal formation volume (cm^3)	$B = -0.02$ Const = 3.72 Beta = −0.49	$B = -0.02$ Const = 3.69 Beta = −0.48	$B = -0.02$ Const = 3.69 Beta = −0.47	$B = -0.02$ Const = 3.69 Beta = −0.48	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Lateral ventricle volume (cm^3)	$B = 0.03$ Const = 6.25 Beta = 0.54	$B = 0.03$ Const = 6.21 Beta = 0.54	$B = 0.03$ Const = 6.25 Beta = 0.54	$B = 0.03$ Const = 6.22 Beta = 0.54	$p < 0.0001$	$p < 0.0001$	$p < 0.001$	$p < 0.001$
Prefrontal cortex volume (cm^3)	$B = -0.36$ Const = 157.65 Beta = −0.33	$B = -0.29$ Const = 156.13 Beta = −0.28	$B = -0.36$ Const = 157.36 Beta = −0.32	$B = -0.30$ Const = 155.89 Beta = −0.27	$p < 0.0001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Gray matter volume (cm^3)	$B = -1.24$ Const = 396.63 Beta = −0.42		$B = -1.23$ Const = 395.21 Beta = −0.39		$p < 0.001$		$p < 0.001$	

L: left hemisphere; R: right hemisphere.

Table 5: Results of the multivariate regression analyses.

Brain structures	Grade of OSA		Grade of liver steatosis	
	L	R	L	R
Caudate nucleus volume (cm^3)	$B = -0.256$ $p < 0.001$	$B = -0.258$ $p < 0.001$	$B = 0.042$ $p = 0.443$	$B = 0.026$ $p = 0.632$
Putamen volumes (cm^3)	$B = -0.124$ $p = 0.002$	$B = -0.125$ $p = 0.001$	$B = 0.061$ $p = 0.225$	$B = 0.083$ $p = 0.144$
Globus pallidus volume (cm^3)	$B = -0.173$ $p < 0.001$	$B = -0.171$ $p < 0.001$	$B = 0.092$ $p = 0.013$	$B = 0.133$ $p < 0.001$
Thalamus volume (cm^3)	$B = -0.407$ $p < 0.001$	$B = -0.419$ $p < 0.001$	$B = 0.007$ $p = 0.912$	$B = 0.044$ $p = 0.559$
Hippocampal formation volume (cm^3)	$B = -0.415$ $p < 0.001$	$B = -0.406$ $p < 0.001$	$B = 0.003$ $p = 0.951$	$B = -0.001$ $p = 0.989$
Lateral ventricle volume (cm^3)	$B = -0.014$ $p = 0.422$	$B = -0.007$ $p = 0.324$	$B = -0.047$ $p = 0.393$	$B = 0.006$ $p = 0.920$
Amygdaloid complex volume (cm^3)	$B = -0.011$ $p = 0.351$	$B = -0.009$ $p = 0.461$	$B = 0.021$ $p = 0.207$	$B = 0.019$ $p = 0.293$
Prefrontal cortex volume (cm^3)	$B = -5.527$ $p < 0.001$	$B = -4.366$ $p < 0.001$	$B = 0.309$ $p = 0.853$	$B = 0.143$ $p = 0.932$
White matter volume (cm^3)	$B = -3.866$ $p = 0.081$		$B = 1.017$ $p = 0.745$	
Gray matter volume (cm^3)	$B = -20.629$ $p < 0.001$		$B = 5.224$ $p = 0.184$	

hippocampal reduction is related to poorer oxygen saturation and higher number of apnea–hypopnea episodes, or poorer blood flow [7, 48]. The influence of OSA on the basal ganglia, particularly on striatal components (caudate nucleus, putamen, and globus pallidus) reduction, shows executive dysfunction, cognitive slowing, working memory deficits, attentional dysfunction, memory retrieval difficulty, impaired language, disturbances (depression, anxiety, and irritability), and impaired procedural memory (for a detailed review, see [49]). The prefrontal cortex is susceptible to

hypoxia according to the fMRI-guided study, as indicated by Zhang et al. [50]. Besides prefrontal, lower blood flow was gained in the anterior cingulate cortex, a part with multiple, but foremost emotion-related functions.

CPAP is the first-line treatment for NAFLD patients with OSA, but the effect of CPAP treatment on liver disease is still controversial and unclear. CPAP treatment may be beneficial to NAFLD patients with OSA independent of metabolic risk factors, but a sufficiently long therapeutic duration longer than three months may be needed to achieve positive effects on the liver enzymes and liver steatosis especially in patients with moderate-to-severe OSA [26, 51]. These data also suggest that CPAP can prevent the progression of NAFLD in OSA individuals. Ng et al. [52] detected significant correlations between hepatic steatosis and markers of severity of OSA but did not show that CPAP alone improves liver steatosis and fibrosis. Weight reduction in obese NAFLD individuals with OSA is associated with an improvement in OSA severity and reduced upper airway collapsibility. Therefore, further research is needed regarding the impact of weight loss and changes in lifestyle and dietary habits on the improvement of liver steatosis and fibrosis in patients with OSA.

The limitations of this study are as follows:

(1) This is a referral, not a cohort study, restricted only to the patients referred to our department and outpatient clinics

(2) A relatively small number of patients with mild and moderate OSA were investigated

(3) Only noninvasive tests were performed for NAFLD

(4) It is limited to newly diagnosed, therapy-naive patients

(5) The diagnoses were dependable on ultrasonographer and sleep doctor's skills and experience

(6) There was an inability to perform functional magnetic resonance imaging because our institution does not possess one

5. Conclusion

Syndrome of OSA worsens the cognitive status in patients with NAFLD. The possible underlying mechanism is the influence on the reduction of cortical and subcortical structures driven by constant apnea/hypopnea episodes, and consecutive hypoxia that initiates the domino process of deteriorating biochemical reactions in the brain.

Data Availability

The data used to support the findings of this study are deposited in the DOI repository.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

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Research Article

Frailty in Nonalcoholic Fatty Liver Cirrhosis: A Comparison with Alcoholic Cirrhosis, Risk Patterns, and Impact on Prognosis

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Background. Physical frailty increases susceptibility to stressors and predicts adverse outcomes of cirrhosis. Data on disease course in different etiologies are scarce, so we aimed to compare the prevalence and risk factors of frailty and its impact on prognosis in nonalcoholic fatty liver (NAFLD) and alcoholic (ALD) cirrhosis. *Patients and Methods*. Cirrhosis registry RH7 operates since 2014 and includes hospitalized patients with decompensated cirrhosis, pre-LT evaluation, or curable hepatocellular carcinoma (HCC). From the RH7, we identified 280 ALD and 105 NAFLD patients with at least 6 months of follow-up. *Results*. Patients with NAFLD compared with ALD were older and had a higher proportion of females, higher body mass index (BMI) and mid-arm circumference (MAC), lower MELD score, CRP, and lower proportion of refractory ascites. The liver frailty index did not differ, and the prevalence of HCC was higher (17.1 vs. 6.8%, $p = 0.002$). Age, sex, serum albumin, and C-reactive protein (CRP) were independent predictors of frailty. In NAFLD, frailty was also associated with BMI and MAC and in ALD, with the MELD score. The Cox model adjusted for age, sex, MELD, CRP, HCC, and LFI showed that NAFLD patients had higher all-cause mortality (HR = 1.88 95% CI 1.32–2.67, $p < 0.001$) and were more sensitive to the increase in LFI (HR = 1.51, 95% CI 1.05–2.2). *Conclusion*. Patients with NAFLD cirrhosis had a comparable prevalence of frailty compared to ALD. Although prognostic indices showed less advanced disease, NAFLD patients were more sensitive to frailty, which reflected their higher overall disease burden and led to higher all-cause mortality.

1. Introduction

Pandemics of inactivity and sarcopenic obesity rapidly increase the global burden of NAFLD [1, 2], which is estimated at 25% and is expected to increase substantially until 2030 [3, 4]. To attract more attention of the general public, it has been recently proposed to rename nonalcoholic fatty liver disease (NAFLD) to "metabolic-associated fatty liver disease" (MAFLD) [5, 6]. Slovakia, with 349 cases of decompensated advanced chronic liver disease (ACLD) per 100,000 inhabitants, ranks number one in the world. The leading cause is alcoholic liver disease (ALD), and the fastest-growing cause is NAFLD [7]. Sarcopenia in NAFLD compared to other cirrhosis etiologies lies higher upstream in the disease pathophysiology. Several reports have highlighted the negative impact of sarcopenia in NAFLD and ACLD [8–19]. Although diagnosing sarcopenia according to the European Working Group on Sarcopenia in Older People (EWGSOP2) consensus is indispensable in academic research, it is less convenient in real-life hepatology practice

[20–30]. In contrast, a simple bedside evaluation of muscle strength in ACLD is also predictive of adverse outcomes [31]. The concept of physical frailty, which is defined by the loss of physiologic reserve and increased susceptibility to stressors, was recently translated from geriatrics to hepatology [32–34]. The functional domains that are best validated for quantifying physical frailty are hand grip strength, chair stand speed, gait speed, and balance time. Physical frailty is an independent predictor of prognosis in ACLD along with a model for end-stage liver disease (MELD) and predicts a range of adverse outcomes in liver transplant (LT) candidates and hospitalized patients [35–41]. The prevalence of frailty in patients with ACLD is estimated at 20–35%, and no difference was found between the sexes [35, 40, 42, 43]. Although some reports have suggested a higher prevalence of frailty in NAFLD cirrhosis, few studies are addressing this issue [40, 43]. The aim of our study was therefore to compare the prevalence of physical frailty, risk factors for its occurrence, and its impact on the prognosis of patients with NAFLD and alcoholic cirrhosis.

2. Patients and Methods

The HEGITO7 registry (RH7) operates in the Department of Hepatology, Gastroenterology, and Transplantation (HEGITO), since 2014. The entry criteria for the registry are as follows: signed informed consent, ACLD requiring hospitalization, and event of cirrhosis decompensation, or evaluation for liver transplantation (LT), or hospitalization for hepatocellular carcinoma (HCC) within the Milan criteria. The registry does not include patients hospitalized for elective procedures, or terminal stages of ACLD or HCC, or with a severely limited life expectancy. The registry contains the date of index hospitalization, basic demographics, medical history, cirrhosis etiology and complications (refractory ascites, RA), body mass index (BMI), hand grip strength (HGS, in kg, using the dynamometer Kern MAP80), mid-arm circumference (MAC, in cm), and tricipital skinfold (in mm, using Harpenden type caliper Somet). During hospitalization, laboratory parameters are recorded (blood count, inflammatory, and synthetic liver function markers). MELD-Na score (further referred to as MELD), Child-Pugh-Turcotte score, and time are needed to complete the number connection test (25 numbers). Since 2017, all patients have been evaluated for functional status by measuring the time required to five chair stands without the help of hands and balance time in three feet positions (parallel, tandem, and semitandem). From the measured parameters, we calculated the liver frailty index (LFI) using a web-based online calculator (https://liverfrailtyindex.ucsf.edu). Since 2019, data from other hospitals in the country are being added to the registry using the same protocol.

For the present study, data from two hospitals were available for analysis. The entry criteria were as follows: patients in the RH7 registry who had NAFLD or ALD, which was considered a salient cause of ALCD, complete data on functional parameters at baseline, and follow-up of at least 6 months.

All procedures involving human participants have been carried out according to the ethical standards of the institutional research committee, including the 1964 Helsinki Declaration and its later amendments (http://www.wma.net) or comparable ethical standards. The reported clinical and research activities are consistent with the Principles of the Declaration of Istanbul, as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. All patients signed informed consent before enrolment into the registry, and data acquisition was approved by the local ethics committee: Etická komisia Fakultnej Nemocnice s Poliklinikou F. D. Roosevelta (in English: Ethics Committee of the Faculty Hospital F.D. Roosevelt), address: Etická komisia, FNsP FD Roosevelta, Nám. L. Svobodu 1, 975 17 Banská Bystrica, Slovakia, on May 21st, 2014.

Due to nonnormal data distribution, numerical parameters are presented as medians and 25–75 percentiles, while proportions are given as numbers and percentages. For comparison of numerical parameters and proportions, we used the Mann–Whitney and chi-square tests, respectively. Missing values were treated as missing and were not accounted for in statistical models. Definition of frailty was adopted according to Lai et al. 2017 (LFI > 4.5) and by calculating the 80th percentile of LFI in our entire cohort of 385 patients (LFI > 5.2). To compare factors associated with frailty between the two etiologies, we constructed linear and multivariable models. Dependent variables were either numerical LFI or categorical frailty (LFI > 4.5). In either case, we used a backward regression model to select covariates independently associated with frailty according to the p value < 0.05.

After discharge from the hospital, patients were followed during preplanned visits after one, three, and six months. Events during follow-up were coded on the day of liver transplantation, death, or censored after more than 6 months. Survival status was verified in the national registry of deceased inhabitants. To clarify the effect of frailty on the prognosis in both groups, we constructed a Kaplan–Meier survival curve and performed a log-rank test (Figure 1). Furthermore, we used the Cox model to determine the relative hazard of death or LT during follow-up in NAFLD compared to ALD patients. For inherent differences in some prognostic variables between the study groups, we adjusted the model for age, sex, MELD score, C-reactive protein (CRP), HCC, and LFI (Figure 2). The results of the model with risk ratios (HR), 95% confidence intervals (CI), and p values are shown in the forest plot (Figure 3). To explore the sensitivity of the hazard ratio for death/LT to the rise of LFI, we added the hazard ratio of NAFLD/frailty to the model. This approach allowed us to quantify the difference in sensitivity to the rise in LFI between patients with NAFLD and ALD.

Statistical analysis has been carried out using the *R* software (R foundation for statistical computing, http://www.r-project.org), *R* Studio (v.1.2.5033, RStudio Inc. for macOS) with the EZR plugin, and MedCalc (MedCalc Software Ltd, Ostend, Belgium).

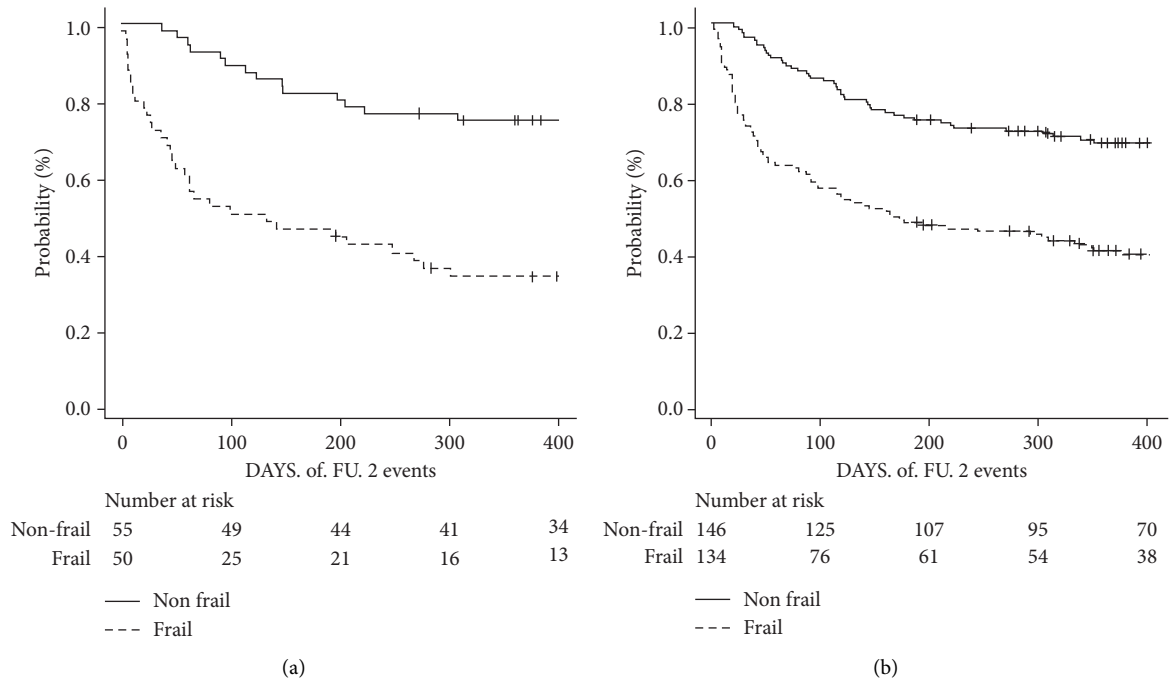

Figure 1: Kaplan–Maier transplant-free survival probability curves and a log-rank test by frailty status, solid line LFI ≤ 4.5, and dotted line LFI > 4.5, in alcoholic cirrhosis (right pane) and NAFLD cirrhosis (left pane), $^{*}p < 0.0001$. (a) NAFLD cirrhosis*. (b) ALD cirrhosis.

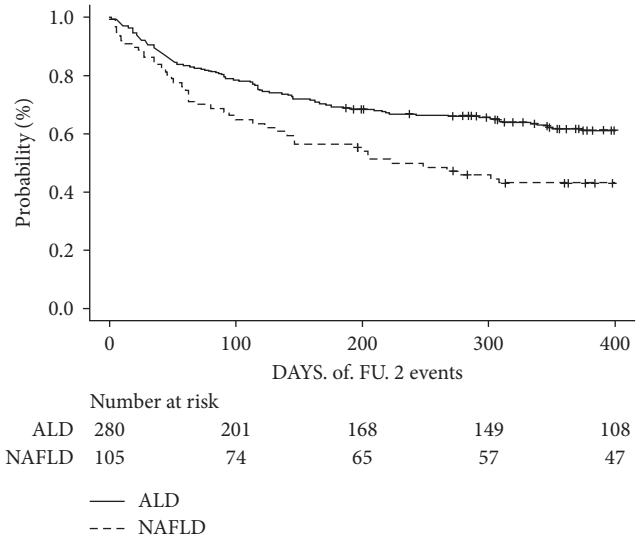

Figure 2: Adjusted Cox model for the probability of transplant-free survival in NAFLD cirrhosis (dotted line) and ALD cirrhosis (solid line). NAFLD HR = 1.9 (95% CI 1.31–2.7).

3. Results

From the registry which at the time included 1221 patients, we identified 385 eligible patients who met the entry criteria. Among them, 280 and 105 patients had alcoholic and NAFLD etiology, respectively. Patients with NAFLD were significantly older and had a higher proportion of females, higher BMI, MAC, and the triceps skinfold (Table 1). Functional parameters such as hand grip strength, chair stands per second, or balance time did not differ between the groups. The LFI was numerically lower in NAFLD patients, but the difference was not statistically significant. Also, NAFLD patients had better baseline parameters of synthetic liver function, MELD score, Child-Pugh-Turcotte score, and lower markers of systemic inflammation (white blood cells, CRP). NAFLD patients also had a higher proportion of cases with hepatocellular carcinoma (HCC, 17.1 vs. 6.8%, $p = 0.002$) and a lower proportion of refractory ascites.

Due to inherent sex-related differences in body composition, we also compared both groups according to sex (Table 2). No difference in nutritional parameters, inflammatory markers, or synthetic liver function between NAFLD and ALD in both sexes was observed. However, LFI was significantly lower in NAFLD men compared to ALD men, but we did not find similar differences in women.

We investigated potential risk factors for frailty separately for NAFLD and ALD in two models. In NAFLD patients, logistic regression yielded the following independent predictors of frailty (LFI > 4.5): male sex (OR = 0.31, 95% CI 0.12–0.816), BMI (OR = 1.16, 1.04–1.28), MAC (OR = 0.79, 0.68–0.91), and CRP (OR = 1.04, 1.01–1.06). In ALD patients, it was age (OR = 1.09, 1.05–1.12), male sex (OR = 0.47, 0.25–0.87), MELD score (OR = 1.11, 1.05–1.16), and the serum albumin (OR = 0.93, 0.89–0.98) (Table 3). The linear model yielded four independent LFI predictors throughout the patient cohort: age, sex, serum albumin, and the CRP. Besides, body mass index and MAC were other predictors of LFI in NAFLD patients and MELD scores in ALD patients (Table 4).

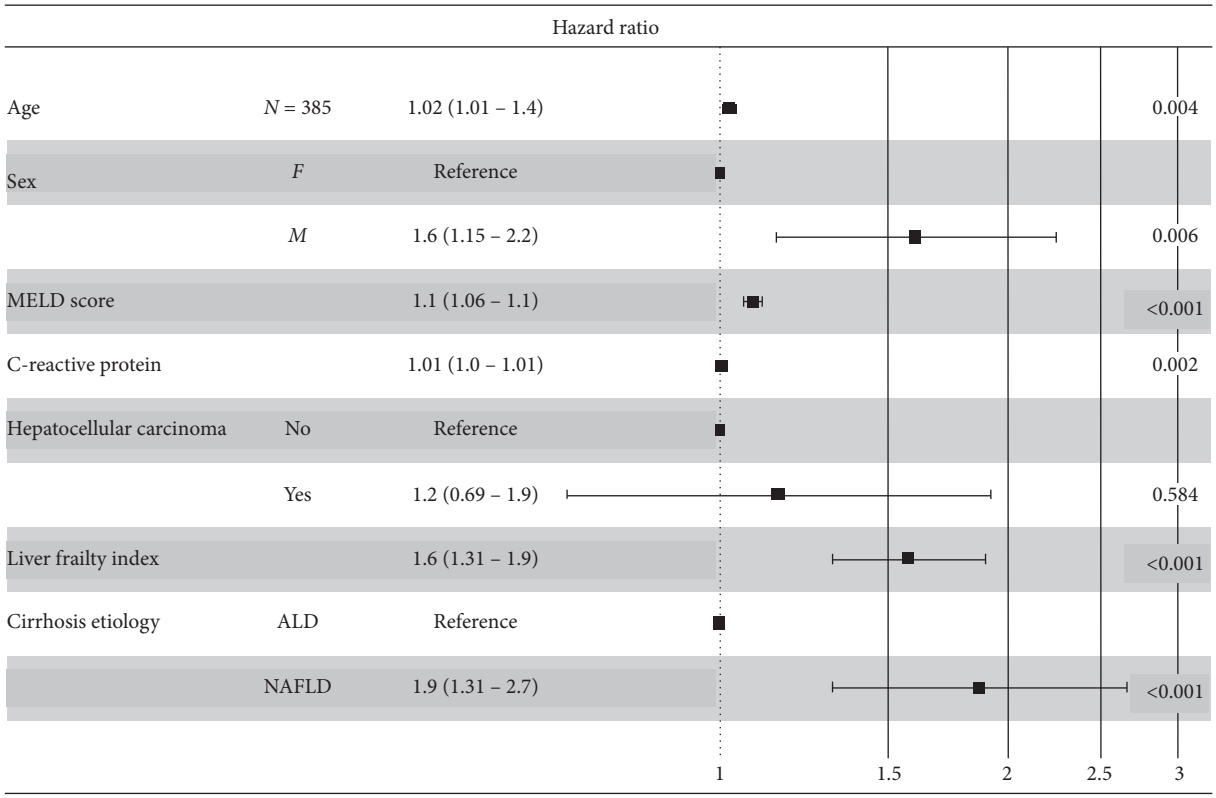

Figure 3: Forest plot of the adjusted Cox model for the predictors of death or LT from the RH7 cirrhosis registry (ALD = 280, NAFLD = 105), events $n = 188$, global p value (Log-rank) <0.0001, AIC = 1072, and concordance index = 0.74.

During follow-up, death or LT occurred within 30, 90, and 180 days in 14.3%, 26.8%, and 37.9% of ALD patients and 13.3%, 27.6%, and 35.2% of NAFLD patients, respectively, with no statistically significant differences between groups. Liver transplantation was carried out in 14 (5.0%) of ALD and 7 (6.7%) of NAFLD cases. The probability of transplant-free survival in both groups stratified according to frailty is displayed in Figure 1. The risk of death or LT was significantly higher in frail compared to nonfrail patients in both groups ($p < 0.001$). In the Cox model that predicts transplant-free survival after adjustment for age, sex, MELD, CRP, HCC, and LFI, NAFLD disease etiology was an independent predictor of death/LT (Figure 2, OR = 1.88 95% CI 1.32–2.67, $p < 0.001$). Forest plot with details of the model is displayed in Figure 3. The model also showed that the HR for death or LT for NAFLD etiology was more sensitive to the rise in LFI compared with ALD disease etiology (HR = 1.51, 1.05–2.2).

4. Discussion

Our study provides evidence that first, frailty substantially increases mortality in patients with cirrhosis of both etiologies. Second, the LFI retains its prognostic power with cutoffs validated in the original study [44, 45]. Third, NAFLD etiology increases the risk of death compared to ALD. Fourth, the impact of frailty on mortality appears to be stronger in NAFLD than in ALD patients.

Upon admission to the hospital, patients with NAFLD and ALD showed a similar prevalence of frailty, indicating a comparable susceptibility to incoming stressors. The observed differences in age, sex, and nutritional status between the groups reflected the differences in the natural history of the disease. In Central Europe with a high prevalence of cirrhosis [1, 46], the median age of ALD cirrhosis at its diagnosis is usually in the mid-fifties [47, 48]. In NAFLD cirrhosis, due to different pathogenetic factors, progression to cirrhosis appears to be slower [49]. Also, the NAFLD cirrhosis outbreak in Central Europe is delayed compared to Western Europe or the USA owing to the later adoption of the Western lifestyle and stronger cultural ties to alcohol. In the region, comprehensive data on the epidemiology and demography of NAFLD cirrhosis are still lacking. However, our data are compatible with some studies from other regions. Sanyal et al. reported a lower incidence of refractory ascites and lower MELD/CTP scores in 150 patients with nonalcoholic steatohepatitis (NASH) cirrhosis compared with HCV cirrhosis. Also, the rate of decompensation and cirrhosis progression was lower in NAFLD patients [50]. In contrast to other previously reported cohorts of NAFLD cirrhosis [51, 52], our study reports data from the registry of hospitalized patients with decompensated disease. In the literature, data on the outcome of decompensated NASH cirrhosis compared with ALD cirrhosis are scarce. One of the studies reported that once the cirrhosis decompensated, the overall survival and liver-related mortality were similar

Table 1: Summary statistics and characteristics of the study groups, a comparison of NAFLD cirrhosis and alcoholic cirrhosis patients.

N	Group	Alcoholic cirrhosis $N = 280$	NAFLD cirrhosis $N = 105$	p value
Age, years		56.91 (48.56, 63.00)	62.26 (55.71, 67.13)	<0.001
Sex, n (%)	Female	85 (30.4)	49 (46.7)	0.004
	Male	195 (69.6)	56 (53.3)	
Body mass index, (kg/m^2)		25.96 (23.06, 29.77)	28.63 (25.34, 34.88)	<0.001
Obese, n (%)		68 (24.3)	44 (41.9)	0.001
Mid-arm circumference (cm)		26.00 (23.00, 29.00)	29.00 (25.00, 33.00)	<0.001
Tricipital skinfold (mm)		9.00 (6.20, 15.10)	14.00 (7.80, 21.40)	<0.001
Mid-arm muscle area (cm^2)		40.23 (33.01, 48.31)	43.92 (35.25, 57.90)	0.002
Hand grip strength (kg)		22.55 (15.88, 29.85)	23.13 (15.83, 29.86)	0.945
Low hand grip strength, n (%)		198 (70.7)	67 (63.8)	0.217
Chair stands (s)		0.36 (0.27, 0.43)	0.39 (0.30, 0.48)	0.167
Chair stands categories, n (%)	Normal	23 (8.2)	16 (15.2)	0.133
	Low	159 (56.8)	57 (54.3)	
	Unable to stand	98 (35.0)	32 (30.5)	
Equilibrum total time (s)		30.00 (20.00, 30.00)	30.00 (24.58, 30.00)	0.143
Equilibrum categories, n (%)	Normal	165 (58.9)	68 (64.8)	0.498
	Low	77 (27.5)	27 (25.7)	
	Unable to stand	38 (13.6)	10 (9.5)	
Liver frailty index (LFI)		4.48 (3.97, 5.04)	4.28 (3.81, 4.87)	0.061
Frailty, LFI > 80th percentile, n (%)		60 (21.4)	14 (13.3)	0.082
Frailty, LFI > 4, 5		134 (47.9)	50 (47.6)	1.00
Serum bilirubin (umol/l)		50.0 (26.51, 137.05)	26.2 (18.8, 76.75)	<0.001
Serum albumin (g/l)		28.90 (24.00, 33.00)	29.00 (27.00, 35.00)	0.018
Serum creatinine (umol/l)		77.90 (59.00, 113.00)	79.00 (63.00, 113.00)	0.868
C-reactive protein (mg/l)		16.23 (6.94, 40.20)	10.84 (4.99, 22.79)	0.010
White blood cells ($*10^9$/l)		7.20 (4.88, 11.03)	5.80 (3.80, 7.50)	<0.001
MELD-Na score		18.91 (14.00, 24.00)	15.00 (11.00, 19.00)	<0.001
Child-Pugh-Turcotte score		10.00 (7.00, 11.00)	8.00 (7.00, 10.00)	<0.001
Hepatocellular carcinoma, n (%)		19 (6.8)	18 (17.1)	0.002
Refractory ascites, n (%)		89 (33.1)	23 (22.3)	0.044
Number connection test, n (%)	Normal	37 (13.2)	13 (12.4)	0.778
	60–90	69 (24.6)	28 (26.7)	
	90–120	70 (25.0)	29 (27.6)	
	>120	83 (29.6)	25 (23.8)	
	Not done	21 (7.5)	10 (9.5)	
Event during follow-up, n (%)	None, LT, death	145, 14, 121 (51.8, 5.0, 43.2)	52, 7, 46 (49.5, 6.7, 43.8)	0.771
Mortality	30 days	40 (14.3)	14 (13.3)	0.87
	90 days	75 (26.8)	29 (27.6)	0.898
	180 days	106 (37.9)	37 (35.2)	0.723

for both etiologies [53]. In the second study, authors reported lower liver-related mortality in NAFLD cirrhosis [54], but once the cirrhosis is decompensated, liver-related mortality was the leading cause of death.

An explanation for the principal findings may lie in the equation: frailty × burden = outcome. Since the prevalence of frailty was comparable, the difference in the outcome would imply the difference in the burden. Baseline characteristics in NAFLD patients show an additional five years in age and only partially reflect a higher disease burden. Although their liver disease burden was more favorable compared to ALD, they had a higher prevalence of HCC. In this study, however, only initial stages of HCC were included, and the presumed impact of HCC on mortality was not confirmed. Even though we adjusted our model for all known confounders, we did not adjust for all comorbid conditions, since our registry does not contain such data. Thus, it is conceivable that NAFLD etiology per se is a composite surrogate of the burden that metabolic syndrome with its extrahepatic manifestations implies on ACLD patients [55] and that baseline disease characteristics do not reflect the overall disease burden. Once frailty has arisen, it reflected a profound effect of the burden of all diseases: the liver-related burden and the burden of comorbid conditions. Similar findings have also resonated in some previous reports among LT candidates. Here, NAFLD patients were three times less likely to be listed for LT compared with patients with viral hepatitis, but they were more likely to die from their liver disease rather than their comorbid conditions [56, 57]. In our small volume liver transplantation center, the reduced chance of enrolling NAFLD patients on the waiting list has not been confirmed. However, a higher likelihood of dying from liver disease was compatible with our results (see limitations paragraph). In contrast, ALD patients initially present with a more pronounced systemic inflammation

Table 2: Sex-specific summary statistics and characteristics of the study groups, comparison of alcoholic and NAFLD cirrhosis.

		Men			Women		
n	Group	Alcoholic cirrhosis $N = 195$	NAFLD cirrhosis $N = 56$	*p* value	Alcoholic cirrhosis $N = 85$	NAFLD cirrhosis $N = 49$	*p* value
Age, years		58.00 (48.88, 63.03)	62.47 (57.50, 67.14)	0.002	55.36 (47.47, 61.27)	61.97 (53.91, 67.00)	0.006
Body mass index (kg/m^2)		27.04 (23.54, 30.41)	29.54 (26.62, 33.58)	0.001	24.24 (21.87, 27.90)	26.78 (24.61, 35.75)	<0.001
Obese, *n* (%)		54 (27.7)	24 (42.9)		14 (16.5)	20 (40.8)	0.003
Mid-arm circumference (cm)		26.50 (24.00, 30.00)	29.00 (26.75, 33.00)	<0.001	24.00 (22.00, 27.00)	27.00 (24.00, 30.00)	<0.001
Tricipital skinfold (mm)		9.00 (6.00, 14.00)	12.20 (7.15, 18.80)	0.016	11.00 (7.00, 17.00)	17.20 (9.40, 24.00)	0.005
Mid-arm muscle area (cm^2)		41.79 (35.67, 50.44)	51.18 (40.69, 63.06)	0.001	34.16 (27.37, 42.50)	39.55 (32.94, 49.88)	0.006
Hand grip strength (kg)		26.27 (21.37, 32.50)	29.07 (24.71, 34.39)	0.018	14.83 (11.07, 17.83)	17.03 (12.43, 19.93)	0.194
Low hand grip strength, *n* (%)		127 (65.1)	30 (53.6)	0.12	71 (83.5)	37 (75.5)	0.267
Chair stands/s		0.36 (0.28, 0.43)	0.44 (0.35, 0.51)	0.004	0.35 (0.26, 0.42)	0.30 (0.22, 0.41)	0.398
	Normal	18 (9.2)	12 (21.4)	0.044	5 (5.9)	4 (8.2)	0.881
Chair stands categories, *n* (%)	Low	110 (56.4)	30 (53.6)		49 (57.6)	27 (55.1)	
	Unable to stand	67 (34.4)	14 (25.0)		31 (36.5)	18 (36.7)	
Equilibrum total time (s)		30.00 (20.00, 30.00)	30.00 (27.07, 30.00)	0.077	30.00 (13.00, 30.00)	30.00 (20.00, 30.00)	0.448
	Normal	118 (60.5)	40 (71.4)	0.353	47 (55.3)	28 (57.1)	0.607
Equilibrum categories, *n* (%)	Low	55 (28.2)	12 (21.4)		22 (25.9)	15 (30.6)	
	Unable to stand	22 (11.3)	4 (7.1)		16 (18.8)	6 (12.2)	
Liver frailty index (LFI)		4.37 (3.91, 4.95)	4.05 (3.59, 4.68)	0.004	4.67 (4.15, 5.40)	4.65 (4.03, 4.96)	0.590
LFI > 80th percentile = 5.2, *n* (%)		35 (17.9)	5 (8.9)	0.146	25 (29.4)	9 (18.4)	0.216
LFI > 4.5		87 (44.6)	18 (32.1)	0.124	47 (55.3)	32 (65.3)	0.279
Serum bilirubin (umol/l)		46.8 (24.8, 113.7)	25.0 (19.9, 46.7)	0.001	75.5 (29.3, 164.9)	27.2 (18.3, 124.4)	0.01
Serum albumin (g/l)		28.00 (24.61, 32.00)	31.00 (26.75, 37.25)	0.002	29.00 (24.00, 34.00)	28.90 (27.00, 32.00)	0.989
Serum creatinine (umol/l)		81.00 (62.00, 119.00)	83.00 (66.75, 118.25)	0.433	74.00 (54.00, 99.00)	71.00 (54.00, 94.10)	0.948
C-reactive protein (mg/l)		14.52 (6.83, 34.20)	8.73 (4.16, 17.75)	0.010	25.29 (7.16, 50.33)	14.61 (6.28, 29.14)	0.122
White blood cells ($*10^9$/l)		6.80 (4.80, 10.60)	5.50 (4.00, 7.32)	0.001	8.00 (5.40, 12.56)	6.50 (3.70, 8.10)	0.003
MELD-Na score		18.89 (13.73, 24.00)	14.50 (11.00, 18.50)	<0.001	19.00 (14.00, 25.00)	15.00 (11.00, 20.00)	0.010
Child-Pugh-Turcotte score		9.00 (7.00, 11.00)	8.00 (6.00, 9.00)	<0.001	10.00 (8.00, 11.00)	9.00 (7.00, 10.00)	0.045
Refractory ascites, *n* (%)		68 (36.4)	13 (23.2)	0.076	21 (25.6)	10 (21.3)	0.671
Event during follow-up, *n* (%)	None, LT, death	95, 5, 22 (48.7, 5.1, 46.2)	29, 5, 22 (51.8, 8.9, 39.3)	0.413	50, 4, 31 (58.8, 4.7, 36.5)	23, 2, 24 (46.9, 4.1, 49.0)	0.383

Table 3: Predictive factors of frailty defined by the liver frailty index > 4.5, a multivariate logistic model.

Comparison of NAFLD cirrhosis and alcoholic cirrhosis			
	OR	95% CI	*p* value
Nonalcoholic fatty liver cirrhosis			
Male sex	0.31	0.118–0.816	0.02
Body mass index	1.16	1.04–1.28	0.006
Mid-arm circumference	0.79	0.684–0.907	0.001
C-reactive protein	1.04	1.01–1.06	0.011
AUROC = 0.85; 95% CI 0.773–0.928			
Alcoholic cirrhosis			
Age	1.09	1.05–1.12	<0.001
Male sex	0.47	0.25–0.867	0.016
MELD	1.11	1.05–1.16	<0.001
Albumin	0.93	0.891–0.984	0.01
AUROC = 0.763; 95% CI 0.707–0.819			

Variables in the model: albumin, BMI, tricipital skinfold, serum creatinine mid-arm circumference, male sex, refractory ascites, age, MELD, and CRP.

Table 4: Predictive factors of the liver frailty index in a linear model.

Comparison of NAFLD cirrhosis and alcoholic cirrhosis				
	Estimate	Std. error	*t* value	*p* value
Nonalcoholic fatty liver cirrhosis				
Intercept	5.12	0.656	7.801	<0.001
Age	0.019	0.006	3.134	0.002
Sex, male	−0.27	0.130	−2.08	0.04
Serum albumin, g/l	−0.041	0.011	−3.664	<0.001
C-reactive protein, mg/l	0.006	0.001	6.609	<0.001
Body mass index	0.024	0.011	2.238	0.028
Mid-arm circumference, cm	−0.046	0.017	−2.694	0.008
Multiple R-squared: 0.5034	Adjusted R-squared: 0.4494			
F-statistic: 9.326 on 10 and 92 DF, *p* value: 1.671e-10				
Alcoholic cirrhosis				
Intercept	3.433	0.445	7.707	<0.001
Age	0.026	0.004	5.948	<0.001
Sex, male	−0.326	0.101	−3.206	0.01
Serum albumin, g/l	−0.025	0.008	−3.068	0.002
C-reactive protein, mg/l	0.004	0.001	3.096	0.002
MELD score	0.027	0.007	3.580	<0.001
Multiple R-squared: 0.2756,	Adjusted R-squared: 0.2476			
F-statistic: 9.818 on 10 and 258 DF, *p* value: 6.7e-14				

Variables in the model: albumin, BMI, tricipital skinfold, serum creatinine. mid-arm circumference, male sex, refractory ascites, age, MELD, and CRP.

and jaundice. Once they begin to abstain, they receive treatment for alcoholic hepatitis and/or systemic antibiotics, and their condition usually improves substantially. Thus, their initial disease characteristics often overestimate the severity of their ALD [58].

Physical frailty assessment using LFI has proven to be a quick and easy tool suitable for the cirrhosis registry. The LFI independently predicted mortality in both cirrhosis etiologies. Our study thus supports in real-life the sustainability of this tool in the context of a resource-limited healthcare system. Our results also validate the diagnostic LFI cutoff of 4.5 in the population of nonwaitlisted patients while retaining its predictive value derived from the original study [44]. But in this study, contrary to our findings, waitlisted NAFLD patients had a higher prevalence of frailty compared to other etiologies [40]. One possible explanation would be in the timing of frailty investigations. Is it likely that ALD patients on the waiting list had recovered from the toxic effects of alcohol and its systemic inflammatory complications.

Our study explores different predictors of frailty in ALD and NAFLD cirrhosis. Age, sex, CRP, and albumin were identified as risk factors in both groups. Higher serum bilirubin concentrations in ALD drove the MELD score high and were likely related to recent alcohol consumption and alcoholic hepatitis. Alcohol has a profound toxic effect on muscle function [15], and once the consumption is stopped, muscle function may improve. In contrast, frailty in NAFLD patients was positively associated with BMI following the previously confirmed effect of obesity on muscle mass and function [10]. Besides, MAC and subcutaneous fat are established indicators of nutritional status. Thus, higher BMI

and lower nutritional status appeared as additional factors exacerbating frailty in NAFLD. The quick reversibility of such conditions is currently questionable and should be subjected to further research. The role of subcutaneous fat, particularly in women, has been described as a stronger predictor of prognosis compared to muscle mass [22]. Although LFI calculation is adjusted for sex, females in our study had a higher risk of frailty. Hence, our data support the assessment and interpretation of body composition and functional status only according to sex. It is beyond the scope of this study to discuss sex-related issues, but it provides complementary data to previous studies on liver transplantation candidates [25–27, 32–35].

Our study has several strengths. Our direct comparison of the two most important etiologies of cirrhosis is rather unique. Due to the recent introduction of LFI as a tool for diagnosing physical frailty in cirrhosis, there is a paucity of data among hospitalized patients [59]. Our study has several limitations. RH7 registry data are limited by the lack of an exhaustive list of comorbidities. A relatively low number of NAFLD cases do not provide sufficient statistical power to address the impact of all such comorbidities. Contrary to our report, some previous studies reported liver-related mortality and not all-cause mortality. Thus, since liver-related mortality could only affect a subgroup of patients, the exact explanation of the increased all-cause mortality in NAFLD patients cannot be provided with confidence. However, when confronted with decisions on patients' management, all diseases need to be taken into account, and our study brings evidence that LFI appears to reflect that. Contrary to the previous studies addressing NAFLD etiology, we did not collect enough computed tomography (CT) results to enrich the muscle mass analysis as suggested by the EWGSOP2 guidelines. However, this limitation is not exceptional in the literature and highlights the advantage of the real-time availability of LFI in the daily practice of many healthcare settings.

5. Conclusions

Our study provides a unique insight into the differences between NAFLD and ALD cirrhosis in hospitalized patients with decompensated disease. Despite older age and a higher proportion of women, NAFLD patients showed a lower liver disease burden and a higher prevalence of HCC. Frailty was equally prevalent and drove all-cause mortality up in both groups. Age, female sex, serum albumin, and systemic inflammatory markers were risk factors for frailty in all patients. Besides, body mass index and MAC were other risk factors of frailty in NAFLD and MELD scores in ALD patients. Frailty and NAFLD demonstrated an independent effect on the risk of death or liver transplantation. Also, NAFLD patients compared to ALD had increased all-cause mortality. Having a higher sensitivity to frailty due to the overall disease burden and lower potential for improvement, management of frailty in NAFLD cirrhosis appears particularly challenging and requires an individualized approach. To improve the prognosis of these patients, we need more interventional studies with clinical endpoints.

Data Availability

The data from the RH7 registry used to support the findings of this study are owned by the Department of Hepatology, Gastroenterology, and Transplantation and will be available on the Mendeley Data repository. The link will be provided from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Review Article

NAFLD and Infection, a Nuanced Relationship

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The prevalence of nonalcoholic fatty liver disease (NAFLD) has increased significantly over the last few decades mirroring the increase in obesity and type II diabetes mellitus. NAFLD has become one of the most common indications for liver transplantation. The deleterious effects of NAFLD are not isolated to the liver only, for it has been recognized as a systemic disease affecting multiple organs through protracted low-grade inflammation mediated by the metabolic activity of excessive fat tissue. Extrahepatic manifestations of NAFLD such as cardiovascular disease, polycystic ovarian syndrome, chronic kidney disease, and hypothyroidism have been well described in the literature. In recent years, it has become evident that patients suffering from NAFLD might be at higher risk of developing various infections. The proposed mechanism for this association includes links through hyperglycemia, insulin resistance, alterations in innate immunity, obesity, and vitamin D deficiency. Additionally, a risk independent of these factors mediated by alterations in gut microbiota might contribute to a higher burden of infections in these individuals. In this narrative review, we synthetize current knowledge on several infections including urinary tract infection, pneumonia, *Helicobacter pylori*, coronavirus disease 2019, and *Clostridioides difficile* as they relate to NAFLD. Additionally, we explore NAFLD's association with hidradenitis suppurativa.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the United States and worldwide. Its prevalence in the USA is about 24%, while it is around 30% in the Middle East and South America [1–3].

The hallmark feature of NAFLD is the aberrant and excessive storage of macrovesicular fat (in >5% of hepatocytes) due to alterations in the homeostatic balance between the fat synthesis and its utilization [1–3]. In some individuals, this seemingly benign fat accumulation in hepatocytes triggers inflammation leading to nonalcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, and/or development of hepatocellular carcinoma. The progression from NAFLD to these more severe entities is multifactorial and depends on an individual's genetic factors, environmental factors, and abnormal activation of the innate immune system [1–5]. Abnormal activation of the innate immune system leads to persistent low-grade inflammation which leads to tissue injury and fibrosis and has an important role in carcinogenesis [1–5].

The liver, in addition to being a vital metabolic organ, also plays a significant role in the human immune system. Liver macrophages (Kupffer cells) and lymphocytes constitute about 20% of total liver cells [6], and they are the first immune cells to process various antigens and pathogens from the gastrointestinal tract. While the role of the immune system is well recognized in the pathogenesis of NAFLD and its complications, it is less known how the presence of NAFLD influences an individual's risk for the development of various bacterial, fungal, and viral infections.

Individuals with NAFLD commonly have one or more elements of metabolic syndrome including obesity, insulin resistance, dyslipidemia, and systemic hypertension. Obesity and type II DM (T2DM) have been previously recognized as risk factors for the development of various infections [7–10].

While patients with NAFLD might have a higher risk for infections due to the concomitant presence of obesity and/or T2DM, some studies have demonstrated an increased risk for bacterial infections independent of the presence of metabolic syndrome in this population [11].

The exact mechanism by which patients with NAFLD might be more prone to the development of infection remains unclear; however, there are several theories. Steatosis might derange sinusoid microcirculation leading to impaired hepatic microbial clearance. Decreased vitamin D levels in NAFLD likely impair innate immunity [12, 13]. There is evidence that NAFLD patients have impaired function of hepatic natural killer cells [14–16]. The impaired function of the Kupffer cells and their aberrant activation might contribute not only to the development of NAFLD but also to an increased risk of infection [17, 18]. Additionally, impaired function of neutrophils in the setting of insulin resistance [19], a higher incidence of small bowel intestinal overgrowth, and dysfunction of the tight junctions of small bowel epithelium causing increased intestinal permeability [20,21] could all further contribute to infection risk in this patient population.

When analyzing the relationship between liver disease and infection, it is important to attend to the nature of the said infection. Untreated infections with hepatotropic viruses such as hepatitis B and hepatitis C (in conjunction with host immune response) cause liver inflammation, fibrosis, and eventually cirrhosis. For these patients, the infection and immunological response of the host are the primary events in the pathogenesis of liver cirrhosis. Conversely, other infections (i.e., fungal and bacterial) are rather the *consequence* of liver cirrhosis and appear dependent on the severity of the disease. It is paramount to recognize that patients with liver cirrhosis have 4-fold higher mortality than patients without cirrhosis [22] and those who develop sepsis have a staggering mortality of up to 75% [23]. Hence, the development of bacterial infection and/or sepsis in the cirrhotic patient has been recognized as a distinct stage in the natural progression of the liver disease [24–26]. Furthermore, bacterial infections are a well-recognized trigger for acute on chronic liver failure, which is also associated with increased mortality [26].

A partial SIRS-like state coupled with negative cultures in 30–50% of infections can make it difficult to differentiate infected from uninfected patients in liver cirrhosis [27]. Cirrhotic patients are also in a state of immune dysfunction combined with a state of excessive activation of proinflammatory cytokines which has been described as "cirrhosis-associated immune dysfunction syndrome" [28]. Immune dysfunction is explained by a decrease in phagocytic activity and a reduction in serum albumin, complement, and protein C activity, along with impaired opsonic activity in serum and ascitic fluid [28, 29]. Additionally, an excessive response of proinflammatory cytokines predisposes to the development of serious complications such as shock, liver failure, renal failure, and death once infection occurs.

The risk of infection appears commensurate with the progression of liver disease from steatosis through cirrhosis. Infection risk likely corresponds to several mechanisms related to the development of portal hypertension, bowel edema, and ascites. Bacterial translocation is the major pathogenetic factor contributing to infections in liver cirrhosis. Alterations to the gut microbiome, increased acid suppression, and increased intestinal permeability in cirrhosis contribute to bacterial overgrowth and enhanced bacterial translocation from the gut to the systemic circulation and ascitic fluid [30]. With the progression of fibrosis in NAFLD, gut dysbiosis has been identified (via gut microbiome-based metagenomic signature) to cultivate more Gram-negative organisms [31]. This is relevant because Gram-negative bacteria are more commonly implicated in bacterial infections with chronic liver disease. Alterations to the microbiome in conjunction with the increased risk of bacterial translocation [20, 21] demonstrate a correlation between the risk of infection and the severity of liver disease.

Additionally, vitamin D deficiency likely has negative impacts on innate immunity [12, 13], thereby contributing to an increased risk of bacterial infections. An inverse relationship has been identified between vitamin D levels and severity of NAFLD [32], which further implicates the progression of liver disease with the risk of infection.

Lastly, a study by Nseir et al. reviewed the association of NAFLD with 30-day all-cause mortality in adult patients admitted with community-acquired pneumonia [33]. The study found that the association was stronger in those with advanced fibrosis (fibrosis score >2) compared to early fibrosis (fibrosis score 0–2), suggesting that disease outcome is correlated with the severity of fibrosis.

The aim of this narrative review is to synthetize data on infection complications in patients with NAFLD, to raise awareness regarding the potential association between these entities, and to promote further research in this area.

2. Methodology

We have used PubMed/Medline database using the following keywords: "NAFLD and infection," "NAFLD and cellulitis," "NAFLD and *Clostridioides difficile* or/and *Clostridium difficile*," "NAFLD and pneumonia," and "NAFLD and COVID 19" to select studies and review articles related to this topic. Articles describing alteration of the immune system as it relates to the pathogenesis of NAFLD were reviewed to a lesser extent as it is not the main goal of this review article. Due to the overall insufficient number of studies (retrospective or prospective) on the risk of infection in people with NAFLD, we were unable to use PRISMA guidelines for a systematic review of literature; rather, we have included these studies in this narrative review.

Due to the recent change in nomenclature, both terms Clostridium and Clostridioides were used in the literature search. A manual review of the references from the articles identified through the database literature search was done to increase the comprehensiveness of the literature review. Articles in languages other than English and Spanish were excluded.

2.1. NAFLD and Bacterial Pneumonia. Pneumonia is a major cause of morbidity and mortality, particularly at the extremes of ages. In the United States, 43,881 (13.4/100 000 population) people passed away from pneumonia in 2019 [34].

A retrospective study by Nseir et al. demonstrated the association between NAFLD and recurrent bacterial infections which appears to be independent of metabolic syndrome [11]. In that study, the incidence of bacterial respiratory tract infections was second only to those involving the urinary tract. Another retrospective review of 141 patients admitted with pneumonia further corroborated the association between pneumonia and NAFLD. In the study, 40.4% of the study group showed evidence of NAFLD compared to 27.6% of patients in the control group [35]. Posttraumatic ventilator-associated pneumonia also appears to be associated with NAFLD. Bailey and Parikh [36] demonstrated an increased prevalence of posttraumatic ventilator-associated pneumonia in NAFLD patients admitted to the critical care unit when compared to patients with other similar risk factors, but without NAFLD. Another retrospective study by Nseir et al. [33] showed an increased mortality in patients with community-acquired pneumonia with NAFLD compared to those without (17% vs. 5.82%). This finding suggests that NAFLD may be related to all-cause mortality in patients with community-acquired pneumonia, an association that became more obvious in those with more severe hepatic fibrosis.

The pathophysiologic mechanisms of pneumonia as they relate to NAFLD have not been clarified in any of the studies. Again, the association of NAFLD with T2DM and obesity is a potential link. Suboptimal functioning of neutrophils in terms of adherence, chemotaxis, phagocytosis, and bactericidal activity is seen in diabetic patients, predisposing them to infections [37], more so with coexisting acidosis [38]. The antioxidant pathways of neutrophils of diabetic patients (e.g., superoxide dismutase) also appear to malfunction [39]. Falguera et al. showed that diabetic patients are more likely to have severe pneumonia with associated pleural effusions as well as higher mortality [40]. Alterations in neutrophil functions described above likely contribute to the predisposition to pneumonia. Although some studies noted no difference in mortality from pneumonia among obese and nonobese patients [41, 42], obese patients are more likely to develop both pneumonia and more severe pneumonia [43]. Adipose tissue is involved in the generation of inflammatory mediators, e.g., leptin and adiponectin [44]. While adiponectin suppresses immunity, leptin is involved in the activation of the immune system. Leptin resistance therefore could potentially be one of the links between obesity and infection. Another mechanism is the participation of adipose tissue in chronic low-grade inflammation [44]. Patients with lean NAFLD [45, 46] are still predisposed to bacterial infections; however, no previous study has compared the risk of bacterial infections between lean and nonlean NAFLD. Given the recent studies that suggest an association of NAFLD with recurrent bacterial infections, there is likely an association which is independent of metabolic syndrome elements. Most likely the association is related to variations in immunity.

2.2. NAFLD and COVID-19. In COVID-19 infections, NAFLD has been found to be a more significant risk factor for hospital admission when compared to age, gender, obesity, or other comorbidities. NAFLD also appears to account for the risk attributed to obesity in COVID-19 which highlights the importance to screen patients with elevated BMI for NAFLD [47].

All human coronaviruses can cause liver injury. Angiotensin-converting enzyme 2 (ACE2) present in liver and biliary epithelial cells acts as the cellular entry receptor for SARS-CoV-2, making the liver susceptible to infection [48]. The cellular transmembrane serine protease 2 (TMPRSS2) is also a critical factor to enable cellular infection by coronaviruses [49]. TMPRSS2 cleaves the SARS-CoV-2 spike protein which allows fusion of the viral and cellular membranes. Systemic inflammation and adverse drug reactions are the main mechanisms of liver injury in severe coronavirus disease. ALT/AST elevation and acute liver injury have been found in 23% and 2% of COVID-19 patients, respectively. Regular monitoring of liver function during hospitalization is important. Both ALT/AST elevation and acute liver injury have been found to be independently associated with adverse clinical outcomes such as ICU admission, mechanical ventilation, and death in COVID-19 patients [50]. When present, it is difficult to differentiate whether the elevation of liver enzymes is due to COVID-19 infection, complications of the disease, or secondary to drug-induced liver injury (DILI).

Several studies suggested that obesity, hypertension, and diabetes greatly increase the risk of severe and prolonged COVID-19 infection [51–53]. As these conditions are commonly present in NAFLD, it is intuitive to see the association between NAFLD and severe COVID-19 disease. It has been postulated that the chronic proinflammatory state associated with these metabolic diseases may play a role, at least in part due to an activation of the renin-angiotensin system [54–56]. This preexisting proinflammatory state seems to favor the cytokine storm, which may result in the multiorgan failure observed in severe COVID-19 [57–59].

The hepatic expression of ACE2 and TMPRSS2 remains unchanged in patients with NAFLD but is downregulated in women, indicating a protective role of estrogens in liver injury caused by SARS-CoV-2 [60–62]. Obese patients are differently affected by T2DM and NAFLD. Obese women with diabetes have unexpectedly lower levels of ACE2 and TMPRSS2 compared to obese normoglycemic women. Conversely, obese patients with NASH show markedly higher expression of these genes, suggesting that advanced stages of NAFLD might predispose individuals to COVID-19 [61].

A study evaluated 202 consecutive patients with confirmed COVID-19 and NAFLD status based on liver ultrasonography. Liver injury was observed in 101 (50%) and 152 (75.2%) patients on admission and during

hospitalization, respectively. Almost all liver injury was mild with a hepatocellular pattern (elevation in ALT). Compared with non-NAFLD subjects, patients with NAFLD had a higher risk of disease progression to severe COVID-19 and longer viral shedding time [63].

Some investigational treatments of COVID-19 have shown uncertain clinical benefit, and their use may be controversial. These consist of antivirals, antibiotics, antifungals, monoclonal antibodies, immune-modulatory agents, anticoagulants, and sedative agents which may be hepatotoxic and cause drug-induced liver injury (DILI). The said treatments may aggravate preexisting liver lesions classically observed in NAFLD including fatty liver, necroinflammation, and fibrosis or trigger the transition of simple fatty liver to NASH [64]. DILI in COVID-19 patients may be worsened by acute heart failure and/or acute kidney injury due to the altered pharmacokinetics of these medications. Hospitalized COVID-19 patients are often polymedicated and at risk for multiple drug-drug interactions and drug-induced adverse events.

While liver injury is not the primary cause of death in COVID-19 patients, hepatic dysfunction can worsen the overall patient's condition, and patients with NAFLD seem to be particularly more vulnerable to these complications. Therapies directed to the treatment of metabolic disease may mitigate the risks from NAFLD.

2.3. NAFLD and Helicobacter pylori. *Helicobacter pylori* is a Gram-negative microaerophilic bacterium that commonly colonizes the stomach in humans. Its prevalence in developed nations is 20% whereas in developing nations its prevalence may be as high as 70%. *H. pylori* is the most important risk factor for chronic gastritis, peptic ulcers, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Growing evidence has linked *H. pylori* to many extra gastrointestinal ailments including obesity, T2DM, ischemic heart disease, and idiopathic thrombocytopenic purpura [65]. There has been a particularly intense focus on the association between *H. pylori* and NAFLD following a 2008 study by Cindoruk et al. which found *H. pylori* 16S rDNA in a liver biopsy of a patient that had NASH. Subsequent research studies have sought to identify any association between *H. pylori* and liver disease in hope of finding an actionable treatment for this growing problem [66]. Some studies have tried to show an association between *H. pylori* eradication and liver fat content and its function. Other studies have tried to investigate the possible function of *H. pylori* in NAFLD pathogenesis particularly as mediated by insulin resistance [67].

While some studies have illustrated a possible association between *H. pylori* and NAFLD, the preponderance of evidence currently available does not support this.

2.4. NAFLD and Hidradenitis Suppurativa. Hidradenitis suppurativa (HS) has been related to NAFLD [68]. At present, there are limited studies looking at the association of HS and NAFLD, but there can be a synergism between bacterial infection and HS leading to this condition.

Hidradenitis suppurativa (HS), also known as acne inversa or Verneuil's disease, is an inflammatory condition of the skin that affects the hair follicle. It usually presents after puberty with painful lesions in the apocrine gland-bearing areas of the body. The most common areas include axillae, inguinal, and anogenital regions [69]. The exact etiology of HS is unknown, but associated factors to developing HS include genetics [70], mechanical stress to the skin [71], smoking [71], hormones [72], obesity [73], and bacteria [74, 75].

It is also considered a systemic inflammatory disease of the terminal follicular epithelium of the apocrine glands, with a prevalence of 0.05% to 4.10%. Interestingly, one cohort study found the prevalence of NAFLD in patients with hidradenitis to be 38.5% [76].

HS was found to be an independent factor for the development of NAFLD after adjusting for classic cardiovascular and steatosis risk factors (OR, 7.75; 95% CI, 2.54–23.64; $P < 0.001$) [68]. In another study, a total of 125 patients with hidradenitis and 120 patients without it were recruited, matched for age, sex, and body mass index (<25 or ≥ 25 kg/m^2). Both groups presented similar proportions of overweight or obesity (89.6% vs. 90%). Patients with HS had a significantly higher prevalence of NAFLD compared with those who did not (57.6% vs. 31.7%, $P < 0.001$). Multivariable analysis confirmed an independent association between HS and NAFLD (odds ratio, 2.79; 95% confidence interval, 1.48–5.25; $P = 0.001$) [77].

Both NAFLD and HS are associated with several other conditions including obesity, dyslipidemia, and insulin resistance [78–80]. After identifying HS as an independent risk factor, it has been hypothesized that the possible explanation for developing NAFLD may be related to the chronic inflammation due to persistent and abnormal secretion of adipokines and several proinflammatory cytokines [81, 82]. Bacterial infection seems to have a role as a synergistic factor in chronic inflammation as postulated by Nikolakis et al. in a review of prospective studies and one retrospective study. While HS is not considered a primary infectious disease, HS is thought to be a skin condition that predisposes to infection, thereafter causing chronic inflammation [75]. The efficacy of targeted antibiotic therapy favors and supports this hypothesis [83–85]. Interestingly, one case-control study found that the microbiome in HS versus non-HS patients is significantly different, and suggests a link between dysbiosis and HS [86].

The proposed mechanism by which HS leads to NAFLD includes the upregulation of proinflammatory cytokines (IL-1b, IL17, and TNF-alpha) [87]. There is also a significant expression of IL-12/Th1 and IL-23/Th17 [88]. Similar to psoriasis, another inflammatory skin condition strongly associated with NAFLD [89–91], the pathogenesis is felt to overlap in the following way: tumor necrosis factor-alpha and interleukins 1, 2, 6, and 17 influence glucose metabolism and insulin sensitivity in hepatocytes and adipocytes causing uncontrolled lipolysis and increased hepatic free fatty acid deposition [78, 92]. Adiponectin, another anti-inflammatory hormone associated with glucose metabolism and insulin sensitivity, is also decreased in patients with HS [81]. Interestingly, to support this pathogenesis, liver biopsies in a

patient with NAFLD have revealed hepatic distribution RNA of the inflammatory cytokines TNF-alpha and the adiponectin with its receptors [47, 93].

2.5. NAFLD and Urinary Tract Infections. Urinary tract infections (UTIs) are rampant in all patient populations, both in the community and in hospitals. A population-based review of laboratory data for residents of the Calgary health region in 2004/2005 showed an annual incidence of 17.5 per 1000 people [94]. Despite the fact that numerous bacteria invade the urinary tract, it is an interaction between individual host factors and the virulence of the organism that eventually determines whether a UTI ensues or not [95].

Established risk factors for UTI include malformations of the urinary tract, female sex, genetic predisposition, and sexual activity [95]. NAFLD has also been identified as a risk factor for bacterial infection [11]. The most obvious pathophysiologic explanation of this is through NAFLD's association with metabolic syndrome.

Previous studies have showed an association between obesity and UTI [96–99]. The association was felt to be more significant in males [96–98] likely due to the positive influence of abdominal obesity on prostatic volume [97]. The association between obesity and urinary tract infection has also been shown to be independent of the association between T2DM and vitamin D deficiency. In premenopausal, nonpregnant women, obesity predisposes not only for a UTI but for recurrent episodes as well [99].

T2DM has long been shown to predispose patients to a number of infections, including those involving the urinary tract [100–102]. Hyperglycemia, diabetic nephropathy, neurogenic bladder, and a malfunctioning innate immune system are all perceived to be contributory [100].

More recent studies are pointing to the fact that NAFLD may be related to urinary tract infections by pathophysiologic mechanisms distinct from those associated with metabolic syndrome. Nseir et al. recently completed a retrospective case-control review of recurrent UTI in premenopausal women admitted to the hospital [103]. In this study, the incidence of NAFLD was higher in the group of patients with recurrent UTI than in the controls (43.5% vs. 21.5%), raising the probability of an association between the two entities. It also showed that patients with recurrent urinary tract infection were more likely to be vitamin D-deficient.

There also seems to be an independent association between NAFLD and vitamin D deficiency [32,104,105], with the degree of deficiency related to the severity of nonalcoholic fatty liver disease [32]. Vitamin D deficiency may independently increase the risk of UTI given that vitamin D is known to stimulate the cathelicidin, an antimicrobial peptide that can be found in the epithelial cells of the urinary bladder [97, 106].

Multiple studies and even meta-analyses have shown an association between nonalcoholic fatty liver disease and urolithiasis [107–109], potentially illustrating another pathophysiologic mechanism for urinary tract infections in this chronic illness. Finally, contributions from defects in both innate and adaptive immunity cannot be ruled out.

More studies (and especially prospective studies) are needed to confirm and further investigate the strength of the association between urinary tract infection and NAFLD. This will potentially open up new avenues for the prevention and management of urinary tract infections in this population of patients [110].

2.6. NAFLD and Clostridioides difficile. While firm evidence is still lacking, it has been postulated that patients with NAFLD might have an increased risk for the development of *Clostridioides difficile* colitis (CDC). Additionally, it has also been postulated that CDC can trigger changes associated with the development of NAFLD [111].

Papic et al. identified NAFLD as an independent predictor for CDC development [111]. In their study, they followed 314 patients of which 83 had NAFLD and 231 were controls, with the NAFLD group demonstrating higher rates of CDC development compared to the controls. Similarly, Bishara et al. [112] recognized that CDC was more frequently diagnosed in patients with higher body mass index (BMI), and they found obesity to be an independent risk factor for CDC development [112].

We postulate that changes in intestinal microbiota in both CDC and NAFLD might be the common denominator and linked with obesity. Since obesity is associated with changes in intestinal microbiota and is commonly found in patients with NAFLD, the association is relatively evident.

The development of CDC is related to derangements in the intestinal microbiota [113], and it has been demonstrated that *Bacteroides* and *Bifidobacterium* play an important role in the mechanism preventing colonization by *C. difficile* [114]. Studies have shown that concentrations of *Bacteroidetes* in the intestines of CDC patients are lower, while intensities of *Firmicutes* and *Proteobacteria* are higher compared to controls [115]. Bearing in mind that obesity has been associated with a relative decrease in the proportion of *Bacteroides* to *Firmicutes* [116], it is not surprising that obese patients might be more susceptible to CDC development.

Similarly, studies on intestinal microbiota in NAFLD patients demonstrated an increase of the phylum *Firmicutes*, *Lactobacillus*, and several genera within the Lachnospiraceae family compared to the healthy individuals [31]. Furthermore, it has been demonstrated that microbiome compositions differ in patients with mild or moderate NAFLD compared to people with advanced fibrosis. By using whole-genome shotgun sequencing of DNA extracted from stool samples, it has been shown that a higher prevalence of *Firmicutes* is present in people with mild or moderate NAFLD, while *Proteobacteria* were dominant microbiota in those with more advanced liver fibrosis [117]. One can draw the conclusion that the risk for CDC might be higher in patients with advanced fibrosis compared to individuals with less severe forms of NAFLD.

Gut microbiota is linked not only to the development of NAFLD but also to its progression. Altered gut microbiota composition and function, together with visceral adipose tissue (VAT) accumulation, results in an imbalance between pro- and anti-inflammatory cytokines. This is considered the

main cause of protracted inflammation in NAFLD and potentially the subsequent increased risk for the development of various infections including CDC.

3. Conclusion

There is strong evidence in the literature that patients with decompensated cirrhosis are at high risk for infectious complications. In patients with NAFLD (but without advanced fibrosis or cirrhosis), however, the evidence is less firm. Studies on this topic are scarce, and of those that exist, many are limited by small sample size or design. The studies are either retrospective or small, single-institution prospective studies. The evidence to date does seem to support an increased risk of infection; however, the extent of this association remains unclear. For certain infections such as pneumonia or UTI, the risk seems to exist primarily through the shared pathophysiology with T2DM and obesity. Hyperglycemia and insulin resistance lead to dysfunctional neutrophils, changes in innate immunity, and possibly vitamin D deficiency. A few studies, however, have demonstrated an increased infection risk in NAFLD even in those without obesity or T2DM. It is possible that the persistent low-grade inflammation associated with the accumulation of fat tissue may change the microstructure of liver tissue and possibly impair the function of liver macrophages (Kupffer cells). In other infections such as CDC, it seems that changes in microbiota in those with NAFLD promote *Clostridioides difficile* colonization and the development of infection. With COVID-19, increased and deregulated cytokine activity has been the main denominator for increased mortality, and patients with NAFLD seem to be particularly vulnerable. Additionally, low-grade inflammation and cytokine derangements are responsible for inflammatory skin changes such as HS. Further research through more rigorous multicenter prospective studies is urgently needed to address the questions regarding this population's risk of infection. As the link is more firmly established, then strategies for treatment and prevention may become evident.

Data Availability

All data are publicly available.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Controlled Attenuation Parameter for Quantification of Steatosis: Which Cut-Offs to Use?

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Chronic liver diseases (CLDs) are a public health problem, even if frequently they are underdiagnosed. Hepatic steatosis (HS), encountered not only in nonalcoholic fatty liver disease (NAFLD) but also in chronic viral hepatitis, alcoholic liver disease, etc., plays an important role in fibrosis progression, regardless of CLD etiology; thus, detection and quantification of HS are imperative. Controlled attenuation parameter (CAP) feature, implemented in the FibroScan® device, measures the attenuation of the US beam as it passes through the liver. It is a noninvasive technique, feasible and well accepted by patients, with lower costs than other diagnostic techniques, with acceptable accuracy for HS quantification. Multiple studies have been published regarding CAP performance to quantify steatosis, but due to the heterogeneity of CLD etiologies, of steatosis prevalence, etc., it had widely variable calculated cut-off values, which in turn limited the day-to-day utility of CAP measurements in clinical practice. This paper reviews published studies trying to suggest cut-off values usable in clinical practice.

1. Introduction

Chronic liver diseases (CLDs) are a public health problem, even if frequently they are underdiagnosed. A study from 2014 estimated that 844 million individuals are affected by CLD, with a mortality rate of 2 million per year [1]. The most frequent CLDs are chronic viral hepatitis, alcoholic liver disease (ALD), and nonalcoholic fatty liver disease (NAFLD) with its progressive variant-nonalcoholic steatohepatitis (NASH). Even if effective treatments are available for chronic viral hepatitis, in NAFLD and NASH this is not the case, an alarming fact considering that the world-vide pooled prevalence of NAFLD is estimated to be 25.24% [2], ranging from approximately 13% in Africa to approximately 30% in Asia and South America. Furthermore, the prevalence of NAFLD is expected to increase since the prevalence of its etiologic factors (obesity, diabetes mellitus, hypertriglyceridemia) is increasing.

Hepatic steatosis (HS) is encountered not only in NAFLD, but also in chronic viral hepatitis, alcoholic liver disease, etc. Several studies demonstrated that HS plays an important role in fibrosis progression, regardless of CLD etiology [3, 4], and that it impairs response to treatment in chronic viral hepatitis [5].

2. Diagnosis of Hepatic Steatosis

Considering all these facts, detection and quantification of HS are imperative, but also a challenge. Detection of HS relies mainly on imaging methods. B-mode ultrasonography is usually the first-line imaging method to detect HS, but it cannot assess the presence of inflammation and it is imprecise to assess steatosis severity, especially mild [6, 7]. Magnetic resonance imaging (MRI) techniques, especially proton density fat fraction (PDFF), are very accurate to detect and quantify HS [8], but they are very expensive and not available enough to be used for assessment of such a large number of patients.

Liver biopsy is considered the gold standard for assessing HS severity, as well as inflammation and fibrosis, when they are present [6, 7]. According to histologic findings, liver steatosis is

classified as absent-S_0 (normal liver), when less than 5% of the hepatocytes have fatty infiltration; mild-S_1, when 5 up to 33% of the hepatocytes present fatty infiltration; moderate-S_2, 33–66% of the hepatocytes with fatty infiltration; and severe-S_3, more than 66% of the hepatocytes with fatty infiltration [6, 7]. However, liver biopsy is an invasive method, poorly accepted by the patients, especially if repetitive, and there are some problems regarding inter-observer variability in assessing the sample, as well as regarding sampling errors [9]. Furthermore, the applicability of liver biopsy to assess such a huge number of patients is highly questionable.

Considering all these facts, noninvasive methods have been developed to assess HS, as well as inflammation and fibrosis (when present). They include biomarkers and imaging techniques [6, 7]. Among the imaging techniques, the controlled attenuation parameter (CAP) feature, implemented on the FibroScan® device, seems the most promising noninvasive test to quantify HS.

3. Controlled Attenuation Parameter (CAP): Technical Data

Vibration-controlled transient elastography (VCTE) (FibroScan®, EchoSens, Paris, France) is an ultrasound-based elastography technique developed more than 15 years ago, firstly used for fibrosis assessment in chronic liver diseases. It is the most validated elastography technique, accepted by international guidelines as a reliable tool to quantify liver fibrosis [10, 11]. VCTE measures the velocity of shear waves generated inside the liver by a mechanical impulse. In CLD, liver stiffness increases with the progression of fibrosis. The stiffer the liver is, the higher the shear waves' velocity.

Several years later, CAP feature was added to the FibroScan® device. It measures the attenuation of the US beam as it passes through the liver. CAP correlates with the viscoelastic characteristics of the liver, dependent in their turn on the quantity of fat droplets in the hepatocytes [12]. CAP measurements can be performed by either the M or XL probes (chosen according to the skin to liver capsule distance), and the results are expressed in decibels per meter (dB/m), ranging from 100 to 400 dB/m [13]. At the beginning, CAP was available only on the M probe of the FibroScan®. Later, it was implemented also on the XL probe developed for obese subjects.

The initial studies regarding CAP showed excellent feasibility-92.3% of cases with only the M probe [13], improved to 96.8% when both M and XL probes have been used [14], also with excellent reproducibility, inter-rater agreement 0.82–0.84 with the M probe [15, 16], but lower with the XL probe, 0.75 and 0.65, respectively [14, 15].

No quality technical parameters have been recommended by the producers to ensure reliable measurements. Therefore, most authors used the quality criteria recommended for VCTE: 10 valid measurements with an IQR/M < 30% [17, 18]. A study published in 2017 recommended as a quality criterion for CAP measurements an IQR < 40 dB/m [19]. When this quality criterion was used, the AUROC of CAP to assess steatosis as compared to liver biopsy increased from 0.77 to 0.9. Another study has set the IQR upper limit at 30 dB/m [8], while another study found no difference in CAP performance when the IQR was ≥30 dB/m or ≥40 dB/m [20]. A recently published study demonstrated that CAP-IQR/M < 0.3 as a quality criterion improves accuracy and feasibility of CAP measurements, performing better than the IQR < 40 dB/m criterion [21].

Regarding the use of M vs. XL probe to assess steatosis grade by CAP, data is still conflicting. In a study performed in a Caucasian population, the cut-off and performance were similar for M vs. XL probe [22], while in a smaller study performed in a Chinese population, cut-off values were higher with the XL probe, but the performance was similar [23]. In a very recent study in Japanese population, cut-off values were higher for the XL probe, but there were no significant differences in accuracy [24].

Several studies demonstrated that CAP measurements are not influenced by the severity of liver fibrosis, nor by the presence of cirrhosis [25–28]. However, several factors have been proven to influence CAP values, among them BMI [29, 30], the presence of diabetes and etiology, especially NAFLD [29], while CAP values higher than 300 dB/m may lead to an overestimation of fibrosis severity by VCTE in patients with lower stages of fibrosis [31].

4. Controlled Attenuation Parameter (CAP): Predictive Value for Steatosis Severity in Individual Studies

Up to date, numerous studies have been published regarding the predictive value of CAP for steatosis severity. We summarized in Table 1 data from studies including more than 100 subjects, with liver biopsy as the reference method, CAP measurements being performed with the M probe (Table 1).

As it can be seen, the performance of CAP for detecting any steatosis ($S \geq 1$) is very good, the AUROC usually being higher than 0.8. In populations with mixed etiology of CLD, the AUROCs remain also high for diagnosing more severe steatosis (S_2 and S_3). However, in NAFLD population, the AUROCs for diagnosing moderate (S_2) and severe (S_3) steatosis decrease, sometimes as low as 0.58 [39], or even 0.37 [38]. Nevertheless, the severity of fat infiltration in NAFLD does not affect prognosis [45], so the important thing is to detect even mild steatosis (S_1), for which CAP is much better than B-mode ultrasonography [46].

The largest individual study assessing the value of CAP for predicting fibrosis severity was published in 2019 by Eddowes et al. [20]. It was a multicenter prospective study that included 450 patients with NAFLD evaluated by CAP/TE and liver biopsy. The AUROCs of CAP to identify patients' steatosis were as follows: for $S \geq S_1$-AUROC of 0.87; for $S \geq S_2$-0.77; while for S_3 it was 0.70. Youden cut-off values were 302 dB/m for $S \geq S_1$, 331 dB/m for $S \geq S_2$, and 337 dB/m for S_3.

The cut-offs also vary a lot among the studies. An explanation could be the relatively small number of patients included in each study, the heterogeneity among groups regarding etiology, overall steatosis prevalence, and also among steatosis severity groups.

Table 1: Performance of CAP (M probe) to diagnose steatosis in patients with CLD, with liver biopsy as the reference method.

Author	No. of patients	Etiology	Prevalence of $S \geq 1$ (%)	$S \geq 1$		$S \geq 2$		$S = 3$	
				Cut-off (dB/m)	AUROC	Cut-off (dB/m)	AUROC	Cut-off (dB/m)	AUROC
Sasso [28]	615	HCV	30	222	0.80	233	0.86	290	0.88
Myers [27]	153	Mixed	65	283	0.81	—	—	—	—
De Ledinghen [25]	112	Mixed	48	215	0.84	252	0.86	296	0.93
Chan [32]	105	NAFLD	97	263	0.97	281	0.86	283	0.75
De Ledinghen [13]	440	Mixed	51.5	—	0.79	—	0.84	—	0.84
Ferraioli [26]	114	Mixed	42.6	219	0.76	296	0.82	—	—
Lupsor-Platon [33]	201	Mixed	45.3	260	0.81	285	0.82	194	0.84
Shen [34]	332	Mixed	42.5	255	0.88	283.5	0.90	293.5	0.84
De Ledinghen [35]	261	NAFLD	100	—	—	310	0.80	311	0.66
Imajo [36]	142 (10 controls)	NAFLD	83	236	0.88	279	0.73	302	0.70
Park [37]	104	NAFLD	91	261	0.85	305	0.70	312	0.73
Naveau [38]	123	NAFLD	81	298	0.81	303	0.58	326	0.37
Siddiqui [39]	393	NAFLD	95	285	0.76	311	0.70	306	0.58
Thiele [40]	269	Alcoholic liver disease	72	290-rule-in 220-rule-out	0.77	328-rule-in 257-rule-out	0.78	339-rule-in 286-rule-out	0.83
Shalimar [30]	219	NAFLD	93.2	285	0.96	331	0.71	348	0.75
Oeda [24]	137	NAFLD	96.3	—	—	264	0.64	289	0.69
Somda [41]	249	Severely obese	84.3	255	0.86	288	0.83	297	0.79
Eddowes [20]	450	NAFLD	88	302	0.87	331	0.77	337	0.70
Baumeler [42]	224	Mixed	62.1	258.5	0.78	282.5	0.83	307.5	0.82
Trowell [43]	217	Mixed	43	278	0.82	301	0.79	—	—
Zeng [44]	173	Liver donors	—	244	0.88	—	0.89	—	—

CAP: controlled attenuation parameter; S: steatosis; AUROC: area under the receiver operating characteristic curve; HCV: hepatitis C virus; NAFLD: nonalcoholic fatty liver disease.

To overcome these shortcomings, meta-analyses have been performed.

5. Controlled Attenuation Parameter (CAP): Predictive Value for Steatosis Severity in Meta-Analyses

The first published meta-analysis included nine studies with 11 cohorts, totalizing 1771 patients with CLD of diverse etiologies [47]. The summary sensitivities and specificities values were 0.78 and 0.79 for $S \geq 1$; 0.85 and 0.79 for $S \geq 2$; 0.83 and 0.79 for S_3, respectively. The HSROCs were 0.85 for $S \geq 1$, 0.88 for $S \geq 2$, and 0.87 for S_3. The median optimal cut-off values of CAP for $S \geq 1$, $S \geq 2$, and S_3 were 232.5 dB/m (range 214–289 dB/m), 255 dB/m (range 233–311 dB/m), and 290 dB/m (range 266–318 dB/m).

The second meta-analysis included 11 studies with 13 cohorts, all of them with high methodological quality, totalizing 2076 patients with CLD of diverse etiologies [48]. The summary sensitivity, specificity, and AUC for $S \geq 1$ were 0.78, 0.79, and 0.86, respectively; for $S \geq 2$, they were 0.82, 0.79, and 0.88, respectively, while for S_3 they were 0.86, 0.89, and 0.94, respectively. Significant heterogeneity was found among the studies for $S \geq 1$ and S_3. CAP cut-of values for $S \geq 1$ ranged from 214 to 289 dB/m, median 238 dB/m; for $S \geq 2$ they ranged from 230 to 311 dB/m, median 259 dB/m, while for S_3 CAP values ranged from 266 to 327 dB/m, median 290 dB/m.

Both meta-analyses above were not able to provide optimized cut-offs with high predictive values due to the limitations of conventional meta-analyses and to the heterogeneity of the included studies, so that a third meta-analysis was performed, this time using individual patient data from 19 studies, including 2735 CLD cases of various etiology, with liver biopsy and CAP measurements [29]. The overall performance of CAP in this meta-analysis was as follows: for $S \geq 1$ the calculated cut-off was 248 dB/m, with 0.68 sensitivity and 0.82 specificity (AUROC 0.82); for $S \geq 2$, the calculated cut-off was 268 dB/m, with 0.77 sensitivity and 0.81 specificity (AUROC 0.86), while for S_3 the calculated cut-off was 280 dB/m, with 0.88 sensitivity and 0.77 specificity (AUROC 0.88).

Another important finding of this last meta-analysis is the fact that, among etiologies, only NAFLD seems to influence CAP values. In other words, NAFLD patients have higher CAP values (by 10 dB/m) as compared with all other etiologies of CLD for the same grade of histologic steatosis [29]. Furthermore, it was calculated that BMI, as well as the

Table 2: Main advantages and weaknesses of CAP/VCTE.

Advantages	Weaknesses
(i) Reproducible method	(i) Expensive equipment
(ii) Well accepted by the patients and thus repeatable assessment possible for follow-up	(ii) Not feasible in patients with ascites
(iii) Good results for noninvasive steatosis assessment in patients with CLD, including NASH	(iii) Increased number of unreliable measurements in patients with high BMI, especially with M probe
(iv) CAP could be used as a screening tool in patients at risk for NAFLD/NASH	(iv) CAP not very accurate to differentiate $S \geq 2$ from S_3
(v) Real-time assessment not only of steatosis but also of fibrosis severity	(v) TE not very accurate to differentiate patients without fibrosis and those with mild fibrosis and patients with moderate vs. mild fibrosis
(vi) Reliable tool for noninvasive assessment of fibrosis, recognized by international guidelines	—
(vii) Results and technical parameters IQR/M available in real time, automatically calculated by the device's software	—

CAP: controlled attenuation parameter; VCTE: vibration-controlled transient elastography.

presence of diabetes mellitus, influences CAP values. Considering these findings, the authors propose an algorithm to correct the measured CAP values, and to apply the cut-offs only after the corrections are made. These correction include deducting 10 dB/m for the presence of NAFLD/NASH, as well as for diabetes mellitus, deducting 4.4 dB/m for each BMI unit over 25 kg/m^2, or adding 4.4 dB/m for each BMI unit bellow 25 kg/m^2.

Finally, a recently published meta-analysis assessed only NAFLD patients (1297 subjects) evaluated by liver biopsy and CAP in nine studies [49]. The mean AUROC, pooled sensitivity, and pooled specificity for diagnosing $S \geq 1$ were 0.96, 0.87, and 0.91, respectively; for $S \geq 2$, they were 0.82, 0.85, and 0.74, respectively, while for S_3 they were 0.70, 0.76, and 0.58, respectively. As observed in individual studies (Table 1), in NAFLD patients the performance of CAP to diagnose steatosis severity decreases as the steatosis progresses. No polled cut-off values have been calculated in this meta-analysis.

6. Controlled Attenuation Parameter, Transient Elastography, and NAFLD/NASH

As mentioned before, the prevalence of NAFLD/NASH is increasing worldwide and in the future will be the main cause of liver-related morbidity and mortality. Considering the high number of patients and the fact that not all patients with NAFLD will develop NASH and liver related events, it is not feasible to try to evaluate all of them by liver biopsy, thus the utility of noninvasive methods. As shown before, individual studies [20, 24, 30, 32, 35–39] and meta-analyses [49] proved the value of CAP for diagnosing steatosis in patients with NAFLD/NASH, even if accuracy decreases with the severity of steatosis [49].

VCTE is the most validated elastographic method for fibrosis assessment in NAFLD/NASH. The cut-off values for different stages of fibrosis vary according to the probe used. For the XL probe (developed especially for obese patients), the cut-offs are as follows: 6.2 kPa for $F \geq 2$, 7.2 kPa for $F \geq 3$, and 7.9 kPa for F_4 [50]. For the M probe the cut-offs are as follows: 7 kPa for $F \geq 2$, 8.7 kPa for $F \geq 3$, and 10.3 kPa for F_4 [51]. In a recent meta-analysis that included 854 NAFLD patients from eight studies, TE had 79% Se and 75% Sp for diagnosing $F \geq 2$ and 85% Se and Sp for diagnosing $F \geq 3$, while for cirrhosis the Se and Sp were 92% [52]. No cut-offs were provided. The accuracy of TE increases with the severity of fibrosis; thus, TE is a very good method to rule in and to rule out cirrhosis.

7. Final Considerations

The ideal diagnostic test should be accurate, available, noninvasive, feasible, inexpensive, and acceptable by the patient. All the data that we presented above suggest that CAP is a feasible test with good accuracy for the detection and quantification of hepatic steatosis, if clinical aspects, such as BMI and presence of diabetes mellitus and of NAFLD/NASH, are taken into consideration. Regarding availability, FibroScan® device is readily available in European countries such as France and even Romania, and, a few years ago, FDA accepted it as a valuable tool to assess fibrosis in the United States. Since it is noninvasive, and it takes only a few minutes to perform, VCTE and CAP are well accepted by the patients. Thus, in some countries, VCTE and serologic markers replaced almost entirely liver biopsy for fibrosis severity assessment [53]. Regarding CAP costs, they are included in those of VCTE assessment of fibrosis and are much lower than of PDFF-MRI, even if with a small loss of accuracy.

Considering all of the above, the rise in NAFLD/NASH prevalence, as well as the steatosis impact on the prognosis of CLD, CAP could be used as a screening tool in patients at risk for NAFLD/NASH (diabetics, obese, patients with metabolic syndrome). Regarding cut-offs to be used, those calculated by the Karlas meta-analysis seem the most robust since they were calculated starting from a large individual data-base meta-analysis and since they take into consideration factors known to influence CAP measurements [29].

The main advantages and weaknesses of CAP/VCTE are summarized in Table 2.

8. Conclusion

Controlled attenuation parameter is a valuable tool to detect hepatic steatosis in day-to-day clinical practice. Cut-off values of 248 dB/m, 268 dB/m, and 280 dB/m, corrected by

BMI and presence of co-morbidities, can be taken into consideration to diagnose $S \geq 1$, $S \geq 2$, and S_3.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Liver and Nonliver-Related Outcomes at 2 Years Are Not Influenced by the Results of the FIB-4 Test and Liver Elastography in a Real-Life Cohort of Patients with Type 2 Diabetes

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Aims. To investigate morbidity and mortality in a real-life cohort of patients with type 2 diabetes (T2D) in relation to prevalence and severity of nonalcoholic fatty liver disease (NAFLD). *Methods*. Patients with T2D were referred for assessment of liver fibrosis by the FIB-4 test and liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE). Liver steatosis was quantified by the controlled attenuation parameter (CAP). These patients were followed until death or censored date. *Results*. Among 454 patients (52% males, mean age 62.5 years, BMI 30.9 kg/m^2), 82.6% was overweight, 77.8% had fatty liver, and 9.9% and 3.1% had LSM and FIB-4 values suggestive of advanced fibrosis, respectively. During the follow-up period of median 2 years, 106 (23%) patients experienced adverse event (11% cardiovascular) and 17 (3.7%) died, whereas no liver-related morbidity or mortality was observed. Independent predictors of adverse outcomes were age and higher platelet count, while FIB-4, LSM, and CAP were not. *Conclusion*. In a cohort of T2D patients, no liver-related morbidity or mortality occurred during 2 years. Our patients probably have low real prevalence of advanced fibrosis which is likely overestimated by LSM ≥ 9.6 kPa. Liver fibrosis may be safely reassessed in the 2 years interval in noncirrhotic patients with T2D.

1. Introduction

Type 2 diabetes (T2D) is among the most prevalent conditions today, affecting almost 10% of the adult population worldwide [1]. It is most frequently accompanied by overweight/obesity which represents the causative factor in majority of the patients through the development of insulin resistance. Together with dyslipidemia and arterial hypertension, these factors constitute metabolic syndrome (MetS) which has been recognized as the leading cause of atherosclerosis and subsequent cardiovascular morbidity and mortality.

Patients with T2D are frequently diagnosed with nonalcoholic fatty liver disease (NAFLD), but this condition has not been well appreciated by international guidelines concerning the diagnostic work-up of diabetic patients. However, in the recent years, a significant body of evidence has been accumulated showing very high prevalence of NAFLD in T2D, a combination associated with poor prognosis in terms of adverse cardiovascular outcomes and higher incidence of extrahepatic malignancy [2, 3]. Among the analysed histological categories, the stage of liver fibrosis has repeatedly been demonstrated as the most important predictor not only of the liver-related but also overall mortality [3]. Interestingly, liver disease does not usually develop to the stage that would compromise overall survival, although live-related outcomes are worse in NAFLD accompanied by T2D as compared to nondiabetic counterparts [4].

For these reasons, active search for the presence and severity of NAFLD in patients with T2D seems intuitive but has not been endorsed by the most relevant international associations for diabetes yet. Possible reasons for this might be the lack of the effective treatment for NAFLD and reliable diagnostic tests [5]. As for the latter, liver biopsy is obviously not the method of choice given its invasiveness and high prevalence of NAFLD, whereas noninvasive diagnostic tests have not been completely evaluated in patients with T2D. Screening for the presence of liver fibrosis should be initiated at the primary care level among at-risk individuals by using simple biochemical tests (such as FIB-4), followed by the second batch tests (using direct markers of fibrosis or elastography) in case of indeterminate results [6]. However, assessment of liver fibrosis in NAFLD might be influenced by the amount of steatosis according to some reports, and the prognostic relevance of these noninvasive surrogates of liver disease in T2D patients has not been completely elucidated [7, 8].

Therefore, the aims of this study were to evaluate liver and nonliver-related outcomes in a real-life outpatient cohort of T2D, in relation to the prevalence and severity of NAFLD as assessed by noninvasive tests. Liver elastography and FIB-4 were tested for their diagnostic and prognostic performances in this cohort of patients.

2. Patients and Methods

2.1. Patients. This investigation was the combination of a cross-sectional study and longitudinal study. In cross-sectional part of the study, we analysed prevalence and severity of NAFLD by using FIB-4 and VCTE among patients with T2D. In the longitudinal part, recruited patients were followed until death or censored date in order to analyse their clinical outcomes in relation to these noninvasive indicators of liver fibrosis and steatosis.

Patients with T2D attending the outpatient diabetic clinic in the tertiary care hospital setting were prospectively assigned to noninvasive assessment of liver steatosis and fibrosis by vibration-controlled transient elastography (VCTE) by the FibroScan device. Three endocrinologists (DR, TM, and SM) referred the first 2 patients (out of around 25 patients having appointment at the respective day) showing up at the outpatient diabetic clinic working twice weekly in the morning from 1 August 2015 to 31 August 2018. Enrolment of the patients was not guided by any risk profiling from the medical history. During 37 months, 468 patients were referred to VCTE. At the diabetic clinic, all patients underwent standardized clinical and laboratory work-up as per the international guidelines [1]. Patients with a history of chronic liver disease of any aetiology other than NAFLD were excluded. In patients referred for the elastographic analysis who had elevated ALT, AST, or GGT, diagnostic work-up was performed in order to rule-out liver disease other than NAFLD (viral or autoimmune hepatitis, autoimmune cholangiopathy, alcoholic liver disease, Wilson's disease, haemochromatosis, and drug-induced liver injury). If any of these aetiologies was confirmed, the patient was excluded from the study.

The FIB-4 test was calculated based upon results of biochemistry determined from a blood sample drawn on the day of evaluation or within the last 3 months and according to the formula that consists of serum values of AST, ALT, platelets, and age of the patient (FIB-4 = (age (years) × AST (IU/L))/(platelets (10^9/L) × ALT (IU/L)$^{1/2}$)) [9]. FIB-4 cutoff values to rule-out (≤1.3) and rule-in (≥2.67) advanced fibrosis were used as suggested by the original study [9].

2.2. Assessment of Liver Fibrosis and Steatosis by Vibration-Controlled Transient Elastography. Liver stiffness measurement (LSM) as the surrogate for liver fibrosis and controlled attenuation parameter (CAP) for liver steatosis was assessed by VCTE with the FibroScan Touch 502 machine by 3 experienced operators (IG, SM, and TB, each having performed > 500 examinations) in fasting patients (at least for 3 hours, usually early in the morning after overnight fasting). The FibroScan probe (M or XL) was chosen according to the automatic probe selection tool embedded within the FibroScan machine. The probe was placed in the intercostal space over the right liver lobe usually in the anterior axillary line, in patient lying in the supine position with the right arm in the maximal abduction. Liver stiffness measurements were performed in the neutral breathing position, during a few seconds of apnoea. Ten LSM per patients were performed, and only those with IQR/median < 30% were considered reliable.

We used dichotomised LSM cutoff values to rule-out (<7.9 kPa) or to rule-in (≥9.6 kPa) advanced fibrosis as suggested by Wong VW et al. [10]. The presence of advanced fibrosis was chosen as the outcome of LSM because this stage of liver fibrosis has been demonstrated and widely accepted as the most relevant prognostic threshold associated with the accelerated development of morbidity and diminished survival in NAFLD [6, 11].

For the assessment of liver steatosis, controlled attenuation parameter (CAP) measurements were performed simultaneously with LSM by the FibroScan Touch 502 device. We used CAP cutoff values as reported by Karla's meta-analysis: 248 dB/m for $S > 0$, 268 dB/m for $S > 1$, and 280 dB/m for $S > 2$ [12]. Despite the reports that the accuracy of CAP declines when its IQR exceeded 40 dB/m, this has not been

confirmed in the recent multicentric study, and therefore, we did not use this criterion as the indicator of reliability [13, 14].

2.3. Follow-Up. In a longitudinal extension of the study, patients were followed until death or censored date (31 December 2018) for the development of liver-related or any other morbidity or mortality by reviewing their medical history in the hospital database or by direct telephone contact with those who did not return for further controls.

Our primary outcome was mortality—liver or nonliver-related, whereas secondary outcome was morbidity, again liver-related and nonliver-related. We considered liver decompensation (jaundice, ascites, portohypertensive bleeding, or encephalopathy), development of hepatocellular carcinoma, or need for liver transplantation as liver-related morbidity. For nonliver-related morbidity, we considered cardiovascular events (acute coronary syndrome, stroke, coronary, or other vascular intervention), infection-related complications that required hospital admission, occurrence of any malignant tumour, and diabetes-related complications requiring hospitalisation (such as diabetic ketoacidosis or hyperosmolar syndrome).

2.4. Statistical Analysis. All statistical analysis procedures were performed using SPSS 24.0 (SPSS, Chicago, IL, USA). Standard parameters of descriptive statistics have been used for determination of baseline characteristics of all variables. All variables were evaluated for normal distribution by using the Kolmogorov–Smirnov test and Student's t-test with correction for unequal variances, where the appropriate was used in order to compare quantitative variables. The chi-square test was used to compare categorical variables. Pearson's or Spearman's nonparametric correlation used was appropriate. Kaplan–Meier curve with appropriate statistical measures was used to assess for survival. We used Cox regression to test the predictive potential of each observed variable for the survival. Variables found to be significant in univariate analysis were used to make multivariate analysis. A 95% level of significance for all tests was accepted for being important.

2.5. Ethical Issues. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's human research committee. Informed consent was obtained from each patient included in the study.

3. Results

We evaluated a total of 468 patients; in 14/468 (2.99%), VCTE measurements were unsuccessful, so a total of 454 patients with T2D (236; 52% males) with mean age (SD) of 62.5 (12) years were recruited. Baseline characteristics of included patients are provided in Table 1. The prevalence of liver steatosis and advanced fibrosis as assessed by CAP, LSM, and FIB-4 was 77.8%, 9.9%, and 3.1%, respectively. In multivariate analysis, factors independently associated with the risk of having advanced fibrosis (LSM ≥ 9.6 kPa) were AST (OR 1.057, 95% CI 1.035–1.080, $p < 0.001$) and cholesterol (OR 0.667, 95% CI 0.467–0.963, $p = 0.026$). Liver steatosis as assessed by CAP did not have a significant impact on LSM (OR = 1.002, 95% CI = 0.997–1.007, $p = 0.45$) readings; although significant but very weak correlation existed in Spearman's analysis (rho 0.189, $p < 0.001$). Independent risk factors for severe steatosis (CAP > 280 dB/m) were BMI (OR 1.093, 95% CI 1.045–1.143, $p < 0.001$), presence of arterial hypertension (OR 1.877, 95% CI 1.046–3.368, $p = 0.035$), ALT (OR 1.029, 95% CI 1.011–1.048, $p = 0.002$), and platelets (OR 0.996, 95% CI 0.992–1.000, $p = 0.043$).

Table 1: Baseline characteristics of patients.

Variable	$N = 454$, median (IQR)/n (%)
Age (years)	64 (56–71)
Male	236 (52%)
BMI (kg/m^2)	30.09 (26.45–34.34)
BMI < 25 (kg/m^2)	79 (17.4%)
BMI 25–30 (kg/m^2)	146 (32.2%)
BMI > 30 (kg/m^2)	229 (50.4%)
AST (IU/L)	22 (18–28)
ALT (IU/L)	24 (18–36)
GGT (IU/L)	29 (20–49)
ALP (IU/L)	71 (60–90)
PLT (×10^9/L)	245 (206–295)
TGL (mmol/L)	1.7 (1.2–2.5)
CHOL (mmol/L)	4.7 (4.0–5.6)
HDL (mmol/L)	1.2 (1.0–1.4)
LDL (mmol/L)	2.7 (2.1–3.5)
HbA1C (mmol/mol)	59 (50–76)
Hypertension	328 (72.2%)
Statin use ($N = 448$)	223 (49.1%)
Skin capsular distance (cm)	2.16 (1.80–2.51)
Use of XL probe	321 (70.7%)
VCTE (kPa)	5.6 (4.4–7.1)
VCTE ≤ 7.9 kPa	368 (81.1%)
VCTE > 7.9 kPa	86 (18.9%)
VCTE ≥ 9.6 kPa	45 (9.9%)
VCTE ≥ 11.5 kPa	33 (7.3%)
CAP (dB/m) ($N = 453$)	310 (256–347)
No steatosis (≤248 dB/m)	101 (22.2%)
Steatosis gr. I (249–268 dB/m)	29 (6.4%)
Steatosis gr. II (269–280 dB/m)	22 (4.8%)
Steatosis gr. III (>280 dB/m)	302 (66.5%)
FIB-4 ($N = 433$)	1.16 (0.84–1.53)
FIB-4 ≤ 1.3	269 (62.1%)
FIB-4 ≥ 2.67	14 (3.1%)

VCTE, vibration-controlled transient elastography; CAP, controlled attenuation parameter; BMI, body mass index; CHOL, total cholesterol; TGL, triglycerides; PLT, platelets.

Comparison of the baseline characteristics between the patients with noninvasive indicators suggestive for the absence of advanced fibrosis (FIB-4 ≤ 1.3; LSM < 7.9 kPa) to those with higher values is presented in Table 2. Additionally, no significant difference existed ($p > 0.05$) in hypertension prevalence and statin use between subgroups presented in Table 2. However, higher frequency of males (96/223; 43.0%) vs. females (67/209: 32.1%) was detected in

Table 2: Comparison of clinical and biochemical characteristics of included patients according to FIB-4 and VCTE values.

	FIB-4	*N*	Mean	SD	*p* value	VCTE	*N*	Mean	SD	*p* value
Age	≤1.3	269	59.61	12.10	<0.001	<7.9 kPa	368	62.38	12.24	0.57
	>1.3	164	66.93	10.33		≥7.9 kPa	86	63.20	10.70	
VCTE (kPa)	≤1.3	269	6.26	3.46	0.04	<7.9 kPa	368	5.17	1.28	<0.001
	>1.3	164	7.04	4.29		≥7.9 kPa	86	12.35	5.16	
CAP (dB/m)	≤1.3	269	301.28	63.36	0.35	<7.9 kPa	368	295.11	66.13	0.024
	>1.3	164	295.07	71.61		≥7.9 kPa	86	312.85	64.54	
BMI (kg/m^2)	≤1.3	268	30.87	7.27	0.09	<7.9 kPa	368	30.24	6.89	0.33
	>1.3	164	29.71	6.64		≥7.9 kPa	85	31.07	7.72	
HbA1C	≤1.3	269	66.30	22.48	0.004	<7.9 kPa	347	63.39	21.48	0.36
	>1.3	157	60.07	19.78		≥7.9 kPa	82	65.82	22.28	
AST	≤1.3	269	21.60	7.92	<0.001	<7.9 kPa	359	23.80	10.98	<0.001
	>1.3	164	36.09	33.55		≥7.9 kPa	86	40.76	44.36	
ALT	≤1.3	269	28.79	18.25	<0.001	<7.9 kPa	359	28.96	21.12	<0.001
	>1.3	164	39.16	41.81		≥7.9 kPa	86	47.69	50.00	
GGT	≤1.3	266	38.86	34.64	<0.001	<7.9 kPa	355	40.26	43.14	<0.001
	>1.3	164	68.39	117.28		≥7.9 kPa	86	95.26	149.04	
ALP	≤1.3	229	76.03	28.74	0.68	<7.9 kPa	307	74.26	27.23	<0.001
	>1.3	147	77.35	29.78		≥7.9 kPa	75	87.07	37.07	
PLT	≤1.3	269	279.70	64.64	<0.001	<7.9 kPa	352	253.37	70.15	0.66
	>1.3	164	208.79	55.99		≥7.9 kPa	82	249.62	72.12	
TGL	≤1.3	254	2.18	1.62	0.32	<7.9 kPa	346	2.40	5.58	0.74
	>1.3	159	2.70	8.07		≥7.9 kPa	79	2.19	1.86	
CHOL	≤1.3	254	4.88	1.35	0.202	<7.9 kPa	346	4.79	1.33	0.57
	>1.3	159	4.71	1.31		≥7.9 kPa	78	4.88	1.38	
HDL	≤1.3	236	1.26	0.71	0.85	<7.9 kPa	317	1.29	0.65	0.006
	>1.3	144	1.28	0.39		≥7.9 kPa	74	1.14	0.36	
LDL	≤1.3	222	2.85	1.13	0.14	<7.9 kPa	294	2.75	1.10	0.32
	>1.3	133	2.67	1.06		≥7.9 kPa	71	2.89	1.10	

VCTE, vibration-controlled transient elastography; CAP, controlled attenuation parameter; BMI, body mass index; CHOL, total cholesterol; TGL, triglycerides.

the subgroup of patients with FIB-4 over 1.3 ($p = 0.024$). Also, higher frequency of XL probe (71/321; 22.1%) vs. M probe (15/133; 11.3%) use for VCTE examination was detected in the subgroup of patients with LSM≥7.9 kPa ($p = 0.023$).

3.1. FIB-4 Score as a Triage Tool with VCTE Serving as the Reference Method. Since the prevalence of advanced fibrosis clearly differed with respect to the noninvasive method used (9.9% by LSM vs. 3.1% by FIB-4), we further explored their interrelationship. We decided to use VCTE as the reference method because it was demonstrated to have much less indeterminate or misclassified cases for advanced fibrosis as compared to FIB-4 (27% vs. 58%). [15] Among 433 patients with available data, FIB-4 values ranged 0.13–7.94 with median of 1.16 (IQR: 0.84–1.53). In 269 (62.1%) patients, FIB-4 was ≤1.3, whereas it was ≥2.67 in only 14 (3.1%) patients (Table 1). In patients with FIB-4 ≤ 1.3, there was 224/269 (83.6%) with VCTE < 7.9 kPal; whereas in patients with FIB-4 > 1.3, there was 37/164 (22.6%) patients with VCTE ≥ 7.9 kPa. More interestingly, among 269 patients with FIB-4 ≤ 1.3, 24 (8.9%) had LSM ≥ 9.6 kPa indicative of advanced fibrosis, and 13 (4.8%) had LSM ≥ 11.5 kPa indicative of cirrhosis; whereas in patients with FIB-4 value > 1.3, there were 21/164 (14.7%) with LSM ≥ 9.6 kPa. As expected, the overall agreement between FIB-4 and VCTE was not statistically significant when assessed with kappa statistics ($\kappa = 0.065$; $p = 0.133$).

Diagnostic performance of the FIB-4 test at the threshold value of 1.3 for advanced (F3) fibrosis as defined by LSM 9.6 kPa in our sample with the prevalence of advanced fibrosis of 10.3% was 46.7% sensitivity, 63.4% specificity, 12.8% PPV, 91.9% NPV, 1.28 LR+, and 0.84 LR−. The AUROC for FIB-4 and for predicting LSM ≥ 9.6 kPa was 0.639, 95% CI = 0.545–0.733, $p = 0.004$. In order to explore if lowering the FIB-4 cutoff value would have improved its diagnostic performance, i.e., decrease the proportion of false-negative patients with advanced fibrosis as determined by VCTE, we chose 1.1 cutoff having 94% NPV in AUROC analysis. However, even with this FIB-4 cutoff, still 11/189 (5.8%) patients had LSM ≥ 9.6 kPa. At this threshold, FIB-4 had sensitivity 73.8%, specificity 45.5%, PPV 12.7%, NPV 94.2%, LR+ 1.35, and LR− 0.58 for advanced fibrosis.

3.2. Survival of Patients in 2 Years Follow-Up. During the median follow-up time of 25 months (IQ range: 9–39), a total of 106 (23.3%) patients experienced an adverse event: cardiovascular in 50 (11%) patients, infection-related in 31 (6.8%), diabetes-related in 22 (4.8%), and oncological in 16 (3.5%), whereas there were no liver-related complications. Seventeen (3.7%) patients died during the follow-up (all deaths nonrelated to liver disease). A Kaplan–Meier curve of

overall survival until any adverse event is shown in Figure 1. Mean time to any adverse event was 36.5 months (95% CI: 35.4–37.5).

We selected a subgroup of patients with the follow-up period of 24 months and more ($n = 374$). A total of 33 patients experienced any adverse event (8.8%): cardiovascular in 17 (4.5%), infection-related in 11 (2.9%), diabetes-related in 7 (1.9%), oncological in 3 (0.8%), and again without liver-related complications. There were 4 deaths (1.1%) in this subgroup of patients, again all nonrelated to liver disease. Mean time to any adverse event was 41 months (95% CI: 40.9–41.5).

3.3. Predictors of Morbidity and Mortality in 2 Years Follow-Up. We performed a univariate Cox regression analysis for the adverse outcome (occurrence of any morbidity or mortality) with all the variables of interest as possible predictors (Table 3). Age, FIB-4, AST, and platelets (PLT) count were significant predictors of adverse outcomes, with borderline significance for CAP and ALT.

The possible influence of different CAP categories on the composite outcome was additionally analysed. Interestingly, the best outcomes in terms of morbidity were observed in the group with most severe steatosis ($X^2 = 9.03$, $df = 3$, $p = 0.029$) (Table 4), whereas no difference in terms of mortality existed ($p = 0.128$). Due to small number of patients in groups with S1 and S2 steatosis, which might have influenced these results, we divided the entire sample into 2 groups according to CAP values ≤280 dB/m and >280 dB/. In Cox regression analysis, we found no effect of CAP at this threshold on survival (HR = 0.85, 95% CI = 0.31–2.29, $p = 0.75$) or occurrence of any morbidity (HR = 0.73, 95% CI = 0.48–1.09, $p = 0.12$).

Variables found to be significant predictors in univariate analysis were additionally included and analysed with stepwise multivariate Cox regression, and only age (HR = 1.046, 95% CI = 1.026–1.066, $p = 0.003$) and platelets count (HR = 1.003; 95% CI = 1.001–1.06; $p = 0.016$) were found to be significant predictors of any morbidity and mortality, while FIB-4 ($p = 0.10$) and AST ($p = 0.64$) were not. This was also true for Cox analysis regarding the mortality—again, age (HR = 1.12, 95% CI = 1.06–1.19, $p = 0.002$) and higher platelet count (HR = 1.007, 95% CI = 1.000–1.013, $p = 0.037$) were significant predictors of mortality, while FIB-4 ($p = 0.28$) and AST ($p = 0.47$) were not.

Then, we divided the sample to three subgroups: into those with platelet count <200 (98; 22.6%), 201–300 (236; 54.4%), and >300 (100; 23.0%) $\times 10^9$/L, whereas for 20 patients (4.4%), platelet count was not available. The sample was then stratified via Kaplan–Meier analysis according to above categories, and although the difference between categories according to survival was not significant ($p = 0.08$), the borderline significance suggests the tendency for higher morbidity and mortality in patients with higher platelet count (Figure 2).

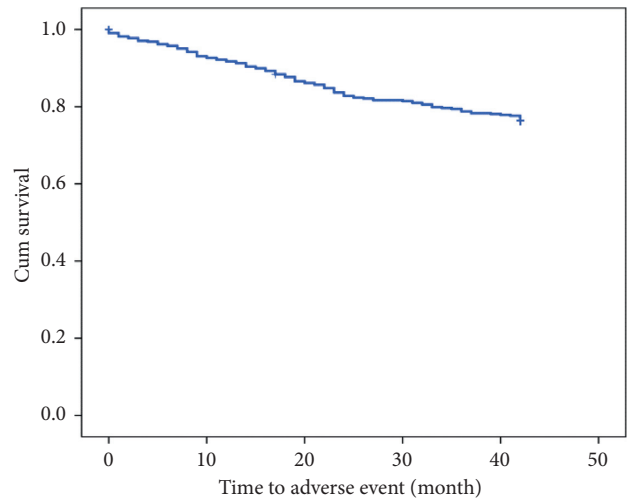

Figure 1: Kaplan–Meier curve of overall survival—time to occurrence of any adverse event during the follow-up period.

Table 3: Univariate Cox regression analysis of predictors of occurrence of adverse events during the follow-up period.

	HR	95.0% CI for HR		p value
		Lower	Upper	
Gender	1.43	0.85	2.41	0.17
Age	1.04	1.01	1.08	**0.02**
BMI (kg/m^2)	1.00	0.97	1.04	0.81
VCTE (kPa)	0.99	0.91	1.07	0.78
CAP (dB/m)	1.00	0.99	1.00	0.05
FIB-4	2.63	1.08	6.39	**0.03**
Hypertension	1.44	0.76	2.74	0.27
Statin	1.35	0.80	2.28	0.26
Smoking	1.84	0.96	3.54	0.07
HbA1C	1.00	0.99	1.02	0.51
AST	0.96	0.92	1.00	**0.04**
ALT	1.02	1.00	1.04	0.05
GGT	1.00	0.99	1.00	0.46
ALP	1.00	0.99	1.01	0.72
PLT	1.01	1.00	1.01	**0.03**
TGL	0.91	0.76	1.10	0.35
CHOL	1.38	0.81	2.35	0.24
HDL	0.70	0.31	1.54	0.37
LDL	0.85	0.48	1.51	0.58

Statistically significant values ($p < 0.05$) are depicted in bold format. VCTE, vibration-controlled transient elastography; CAP, controlled attenuation parameter; BMI, body mass index; CHOL, total cholesterol; TGL, triglycerides.

4. Discussion

This study conducted over the large cohort of patients with T2D reveals high prevalence of overweight/obesity and liver steatosis (both around 80%). Results of FIB-4 and VCTE were not concordant in predicting the proportion of patients with/without advanced fibrosis. Over the follow-up period of median 2 years, no liver-related morbidity or deaths were reported, and therefore, the real prevalence of advanced

Table 4: Influence of the CAP value on composite outcomes (any morbidity or mortality).

			Any morbidity or mortality		Total
			No	Yes	
CAP value (dB/M)	≤248	*N*	67	33	100
		%	67.0%	33.0%	100.0%
	249–268	*N*	21	8	29
		%	72.4%	27.6%	100.0%
	269–280	*N*	15	7	22
		%	68.2%	31.8%	100.0%
	>280	*N*	242	58	300
		%	80.7%	19.3%	100.0%
Total		*N*	345	106	451
		%	76.5%	23.5%	100.0%

$X^2 = 9.03$; $df = 3$; $p = 0.029$. CAP, controlled attenuation parameter.

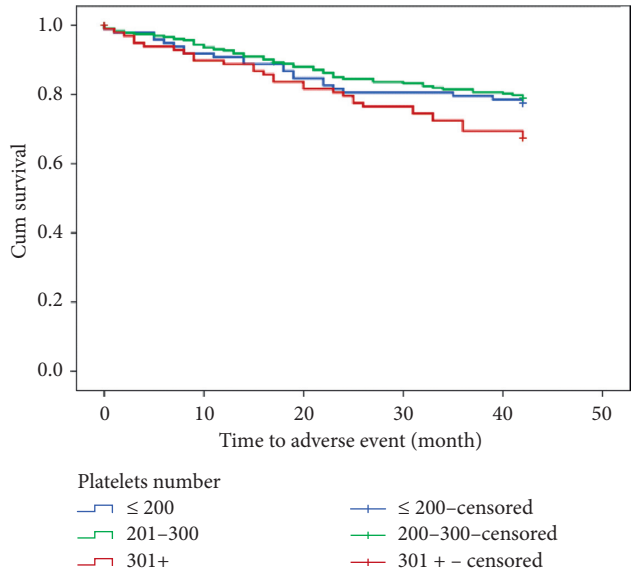

Figure 2: Kaplan–Meier curve of overall survival—time to occurrence of any adverse event during the follow-up period stratified according to platelets number.

fibrosis in this cohort was likely low and overestimated by LSM ≥ 9.6 kPa. Among 23% of patients who experienced adverse outcome, half was caused by cardiovascular events. FIB-4, LSM, and CAP as the noninvasive surrogates of fibrosis and steatosis, respectively, were not predictive for adverse outcomes in the analysed cohort and the period of time.

Diabetes is a very prevalent condition, affecting around 9% of the world adult population [1] and goes hand-by-hand with the epidemics of overweight/obesity. In Europe, around 50% of population is overweight, and almost half of that number is obese [15]. Obesity and the resultant insulin resistance are the important metabolic conditions associated with the development of NAFLD, although several authors argue pointing to the more important pathophysiological role of the fatty liver that facilitates development of insulin resistance and T2D [16]. Whichever is right, people with T2D, especially with obesity and NAFLD share common dysfunction of metabolic pathways and are accompanied by other comorbidities such as dyslipidemia and arterial hypertension, commonly known as metabolic syndrome [17].

This syndrome is associated with increased cardiovascular morbidity and mortality. Patients with fatty liver have relatively good prognosis, and the major determinant of their long-term outcome is the presence of liver fibrosis [2]. Around 1/3 of patients with NAFLD develop fibrosis and are in risk for liver-related morbidity and mortality [18]. Also, these patients are more endangered in terms of cardiovascular and oncological events and mortality [3]. It has been repeatedly shown that the presence of T2D in patients with NAFLD represents risk for progressive course of liver disease, and for vice versa, some conflicting results were published [4, 19].

These are the reasons why we should be interested at evaluating patients with T2D for the presence and severity of NAFLD. For this purpose, noninvasive tests have gained much popularity for being easy to perform, available, painless, and with acceptable accuracy in diagnosing and quantifying liver steatosis and fibrosis. Whereas, the impact of steatosis has not been proven, and fibrosis plays the prominent role on the development of liver-related complications as well as overall morbidity and mortality as already pointed out. According to recent data, steatosis might be present even in the patients with the compensated advanced chronic liver disease (cACLD), and the higher grade of steatosis might be associated with the worse prognosis in terms of decompensation and death [20–22]. For less advanced stages of chronic liver disease, probably the rationale for quantifying liver steatosis is to objectively follow reduction in steatosis while the patient is taking lifestyle measures to correct his/her metabolic abnormalities.

Our cohort of T2D is similar to the other cohorts reported in the literature. Around 80% of them are overweight/obese and 80% has NAFLD, and almost 10% of them have advanced fibrosis according to LSM assessment by VCTE [23]. However, real proportion of advanced fibrosis would have probably been lower if it was assessed histologically, since it has been previously demonstrated that VCTE had only 59% PPV, meaning that at most 6% of our cohort would in fact have advanced fibrosis [23]. This conclusion is furtherly supported by the absence of liver-related events in our cohort during the follow-up. The potential influence of steatosis on LSM readings is rather controversial issue as some reports do and the others do not suggest association between them [7, 8, 14]. Although a weak correlation between CAP and LSM existed, in multivariate analysis, CAP was not independently associated with the risk of having advanced fibrosis in our cohort.

Based on our data, FIB-4 < 1.3 has 92% NPV for ruling out advanced fibrosis in patients with T2D, with marginal improvement of NPV to 94% at lower FIB-4 threshold of 1.1. Our results are in keeping with current evidences claiming high NPV of the similar order of magnitude for FIB-4, but its PPV is suboptimal, and in addition to this, significant number of false-negative cases (8% according to our results) still appears below this threshold [24].

In terms of predictive capability of baseline noninvasive parameters, only age and higher platelets count were

predictive for adverse outcomes in our cohort, whereas other demographic, biochemical (including FIB-4), or elastographic (LSM and CAP) values were not. Our results are in agreement with recently published data from Edinburgh cohort of T2D patients demonstrating suboptimal predictive ability of several noninvasive biochemical indices including FIB-4 which had 11–18% false-negative predictive rate for cirrhosis or HCC at 1.3 cutoff, whereas PPV of 40–46% at 2.67 cutoff value was equally poor [25]. Similarly, LSM did not influence the outcomes, although 10% of patients had liver stiffness reading over the threshold for advanced fibrosis (≥9.6 kPa). However, VCTE in general has much better performances to rule-out than rule-in advanced fibrosis or cirrhosis. Published PPVs for advanced fibrosis at LSM threshold of 9.6 or 9.7 kPa ranges 59–72.4%, whereas PPV for cirrhosis defined at cutoff 11.5 kPa was below 50% in Wong's study and for cutoff 13.6 kPa only 29% in Eddowes' study [10, 14, 23]. In the latter study, optimised cutoff for cirrhosis with 90% specificity was 20.9 kPa, and even at this high threshold, its PPV was only 37%. Therefore, LSM ≥ 9.6 kPa likely overestimated real prevalence of advanced fibrosis in our cohort. Furthermore, only 5 patients had LSM values over 20.9 kPa, and given the low PPV, it might be that in fact no patient had cirrhosis. In addition to probably very small proportion of patients with advanced fibrosis, our results are also not surprising because the follow-up period was relatively short. Bearing in mind that development of liver fibrosis and end-stage liver disease is relatively a slow process, it is not unexpected that no liver-related adverse outcomes were noticed. This may lead to general conclusion that noncirrhotic patients with T2D might be relatively safely followed by VCTE every 2 years. This is in line with the results of the Swedish study on the natural history of NAFLD (from general population, not only diabetics) which demonstrated that it needs at least 2.3 years for the first 10% of patients with advanced liver fibrosis to develop cirrhosis, liver decompensation, or HCC [11]. However, the presence of cirrhosis, when reliably diagnosed, should lead to intensified surveillance for the occurrence of HCC every 6 months by ultrasound according to current recommendations [26]. As for the predictive role of platelets count for the CV morbidity/mortality, this association has already been demonstrated and probably results from higher thrombogenic risk in patients with higher platelet count [27].

This study has limitations. First of all, patients were prospectively included over the long period of time, whereas the follow-up period was relatively short, so we were not able to analyse neither long-term outcomes of patients with T2D and NAFLD nor the potential impact of LSM, CAP, or FIB-4 in this regard. Furthermore, this study lacks liver biopsy to make firm conclusions about the severity of liver disease and the interrelationship between some histological categories and their influence on CAP and LSM. Nevertheless, outcomes were clearly defined and analysed as the occurrence of liver-related or any other morbidity and mortality. There is also an issue of LSM threshold values for various fibrosis grades and current controversy whether the use of the XL probe or CAP value has an impact on LSM measurement. Given the recent evidence, neither the probe type (M/XL) nor the CAP value has been confirmed to influence LSM as assessed by VCTE [14]. Which is the best cutoff value for a certain stage of liver fibrosis may be a matter of discussion because there is no 100% agreement between the studies and authors. We used cutoff values proposed by Wong et al. because most studies published so far referred to these cutoff values [9]. We do not believe that using the different cutoffs would likely change the main messages derived from this research.

In conclusion, T2D patients in this cohort had high prevalence of overweight/obesity and liver steatosis (both around 80%). In this group of patients, FIB-4 as a triage tool has good NPV for ruling-out advanced fibrosis, with marginal improvement at the lower threshold of 1.1. Real prevalence of advanced fibrosis in our cohort was likely low and overestimated by LSM ≥ 9.6 kPa by VCTE. This conclusion is supported by the absence of liver-related events during the follow-up period. Therefore, in the cohort of patients with T2D with probably low prevalence of advanced fibrosis, noninvasive tests for fibrosis were not predictive for adverse outcomes over the analysed period of time, and the same holds truth for the prognostic impact of liver steatosis quantified noninvasively by CAP. Among 23% of patients who experienced adverse outcome, half was caused by cardiovascular events. Patients with T2D could probably be safely monitored for liver-related complications in 2 years intervals, provided that cirrhosis has been reliably ruled-out.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

(i) Real prevalence of advanced fibrosis in our patients with T2D seems low

(ii) LSM ≥ 9.6 kPa by VCTE likely overestimates advanced fibrosis in patients with T2D

(iii) No liver-related morbidity/mortality occurred over 2 years

(iv) FIB-4, LSM, and CAP were not predictive for adverse outcomes

(v) Liver fibrosis may be safely reassessed in 2 years in noncirrhotic T2D patients

Ethical Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Hospital Dubrava, No. 29062015, on June 29, 2015.

Consent

Informed consent was obtained from all individual participants included in the study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ivica Grgurevic conceptualized the study, developed methodology, collected data, wrote the original draft, reviewed and edited the article, and supervised the study. Nermin Salkic developed methodology, performed formal analysis, developed software, wrote the original draft, and reviewed and edited the article. Sanda Mustapic, Kristian Podrug, Dario Rahelic, Tomas Matic, and Viktoria Skurla collected data, investigated the study, and reviewed and edited the article. Tomislav Bokun collected data, investigated the study, reviewed and edited the article, and visualized the study. Srecko Marusic and Ivana Mikolasevic supervised the study and reviewed and edited the article.

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Research Article

Association between Gastroesophageal Reflux Disease and Elastographic Parameters of Liver Steatosis and Fibrosis: Controlled Attenuation Parameter and Liver Stiffness Measurements

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Aim. Our aim was to investigate the association among elastographic parameters of liver steatosis and fibrosis, controlled attenuation parameter (CAP) and liver stiffness measurement (LSM), with gastroesophageal reflux disease (GERD). *Methods*. In this prospective, cross-sectional study, we have evaluated 937 patients with one or more components of the metabolic syndrome who had an esophagogastroduodenoscopy (EGD) due to GERD symptoms. In all patients, a laboratory analysis, an abdominal ultrasound, and FibroScan measurements were done. GERD was defined by EGD. *Results*. The mean body mass index (BMI) of the study population was $30.95 \pm 5.45\,kg/m^2$. The prevalence of increased CAP was 82.6% (774/937). Patients with increased CAP were younger, were more obese, had higher prevalence of hypertension, diabetes, and dyslipidemia, and had higher values of aminotransferases. Similar results of higher prevalence in patients with elevated CAP were observed with GERD, hiatal hernia, and insufficient cardia (defined as deficient or absent closure of the gastric inlet in relation to the esophagus). Additionally, patients with elevated CAP had a higher prevalence of GERD grades B and C in comparison to those without elevated CAP. Consequently, patients who did not have elevated CAP had a higher prevalence of GERD grade A. Even though we have found an upward trend in the prevalence of GERD, hiatal hernia, and insufficient cardia, there was no significant difference between subjects with fibrosis (F) 1-2 and F3-4 stage of fibrosis or F1 and F2-4. In a binary logistic regression, a significant positive association with GERD was obtained for CAP. Furthermore, a significant positive association with hiatal hernia was obtained for BMI and CAP. Finally, a significant positive association with hiatal hernia was obtained with CAP in multivariate analysis. *Conclusion*. To the best of our knowledge, our study is the first to reveal a positive association between CAP as a surrogate marker of liver steatosis and GERD after adjustments for other clinical variables.

1. Introduction

According to data, about 25% of all cancers are in the gastrointestinal tract (GIT), making it the dominant cancer affected site [1]. As it is the case with most human tumors, esophageal carcinoma (EAC) is preceded by premalignant lesion or Barrett esophagus (BE). The main characteristic of BE is abnormal transformation of the squamous epithelium. Gastroesophageal reflux disease (GERD) is one of the most common GIT-related diseases worldwide and one of the

most common indications for visiting gastroenterologists [1–7]. In the context of GERD, reflux of stomach contents into esophagus is responsible for the most common symptoms of this condition: heartburn, regurgitation, and dysphagia. A major concern of physicians who manage patients with GERD is the increased risk of EAC; thus, GERD is the most important risk factor for BE and EAC development [2–6]. The prevalence of GERD in general population is about 30% with an increasing overall, which is not surprising regarding the data that obesity (especially abdominal obesity) and the metabolic syndrome (MetS) are risk factors for GERD development [6–10].

Nonalcoholic fatty liver disease (NAFLD) is an increasingly growing cause of end-stage liver disease (i.e., liver cirrhosis and hepatocellular carcinoma (HCC)) and is the most common cause of chronic liver disease (CLD) today [11]. NAFLD is a clinical syndrome characterized by liver steatosis in individuals with no history of alcohol abuse, comprised of a spectrum of disorders. Histologically, there are few disorders in the context of NAFLD; for the first, there is simple steatosis, then necroinflammatory form called nonalcoholic steatohepatitis (NASH), then fibrosis and advanced fibrosis, and finally, cirrhosis. Normally, HCC is predisposed with the presence of cirrhosis, but in the context of NAFLD, HCC can evolve in non-cirrhotic NAFLD [11–13]. NAFLD is closely connected with the MetS and its individual components, diabetes mellitus type 2 (T2DM), arterial hypertension, obesity, and dyslipidemia [11–13]. The prevalence of NAFLD goes hand in hand with the prevalence of MetS and obesity due to its multisystemic effect; this combination is connected with the most serious health threat responsible for increasing number of chronic kidney diseases, cardiovascular, oncologic, and liver-related morbidity and mortality [12, 14]. In everyday clinical practice, the diagnosis of NAFLD represents a clinical challenge because most of NAFLD patients are asymptomatic. Although it is not the optimal method, liver biopsy (LB) is still the gold standard for the diagnosis and staging of NAFLD. Since around 25% of the population has NAFLD, noninvasive methods are being intensively investigated. The most investigated among elastographic methods is transient elastography (TE). With the help of controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) obtained by TE, we can detect and quantify steatosis and fibrosis [15]. According to a recent study, CAP and LSM are good noninvasive methods for the assessment of steatosis and fibrosis in patients with NAFLD [16].

The relationship between NAFLD and GERD is controversial and published data are conflicting. According to some authors, there is no connection between these two conditions [5], while some others have found that GERD and its symptoms are more prevalent in NAFLD patients [6, 9, 10, 17]. According to our best knowledge, there are no published manuscripts that investigated the association between GERD and elastographic parameters of liver steatosis and fibrosis: CAP and LSM.

Therefore, our aim was to investigate the association among elastographic parameters of liver steatosis and fibrosis, CAP and LSM, with GERD.

2. Patients and Methods

2.1. Patients. In this prospective, cross-sectional study, we have evaluated 1050 patients with one or more components of the MetS who had an esophagogastroduodenoscopy (EGD) due to GERD symptoms (heartburn, regurgitation, and dysphagia) during the 24-month period between January 2018 and December 2019. In all patients, a laboratory analysis, an abdominal ultrasound (US), and FibroScan measurements were done. Patients who signed informed consent forms and were older than 18 years were part of this investigation. Patients with incomplete data, those who refused to undergo TE or US examination, those with significant alcohol consumption (>20 g per day for men and >10 g per day for women), other CLD (viral, metabolic, or autoimmune), celiac disease, and those with secondary causes of fatty liver such as drugs (amiodarone and tamoxifen) were excluded from the final analysis. Additionally, active malignancy, congestive heart failure and valvular heart disease, TE failure, and pregnancy were additional exclusion criteria. Because of these exclusion criteria, 937 patients were included in the final analysis. The Clinical Hospital Rijeka Ethics committee approved this research. Appropriate informed consent forms were signed by all patients. We conducted the research in accordance and agreement with the International Conference on Harmonization guidelines on Good Clinical Practice and with the Declaration of Helsinki.

2.2. Outcomes. The primary outcome of this study was to evaluate the association among elastographic parameters of liver steatosis and fibrosis, CAP and LSM, with the presence of GERD, hiatal hernia, insufficient cardia, and BE. Secondary outcomes were to investigate the association of GERD, hiatal hernia, BE, and insufficient cardia with laboratory, demographical data and elastographic parameters.

2.3. Clinical Assessment. In all analyzed patients, information on medical history and current drugs was recorded as well as demographic (age, sex, smoking, and alcohol consumption) and anthropometric (body mass index (BMI), waist circumference (WC), hip circumference (HC), and upper arm circumference (UAC)) data. Smoking was classified as nonsmoker, ex-smoker, and smoker. In all patients, information regarding the presence of one or more MetS components was analyzed. BMI was calculated as weight (kg)/height (m^2). Hypertension was defined if the average blood pressure (after three repeated measures) was ≥140/90 mmHg, if there was positive medical history, or if the patient was taking anti-hypertensive drugs. Diabetes was defined as a fasting plasma glucose level ≥5.6 mmol/L or previously diagnosed T2DM or use of any hypoglycemic drugs. Dyslipidemia was defined as positive medical history, using of lipid-lowering drugs, or if the serum total cholesterol level was ≥5.2 mmol/L, serum triglyceride (TG) level ≥1.7 mmol/L, serum high-density lipoprotein (HDL) cholesterol level <1.0 mmol/L for male or <1.3 mmol/L for

female, or serum low-density lipoprotein (LDL) cholesterol level ≥3.4 mmol/L.

An extensive laboratory evaluation was done in each patient in the morning hours after overnight fasting at the day of TE examination. Blood samples were collected from the patients to determine the full blood count and serum levels of liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP)), fasting plasma glucose and fasting insulin, lipidogram (total cholesterol, LDL and HDL cholesterol, and triglycerides), renal tests (urea, creatinine), ferritin, serum uric acid, and C-reactive protein (CRP). Apart from a routine laboratory, each patient was screened for viral and other causes of CLD (metabolic and autoimmune). Well-trained nurses were responsible for measurements of anthropometry, blood pressure, and blood sampling.

2.4. Transient Elastography and Ultrasound Examination. Abdominal ultrasound examination was performed by an experienced specialist (gastroenterologist) with the help of Philips Affiniti (PC Best, Netherlands). As mentioned, in all patients TE examination after overnight fasting was done by using FibroScan® 502 Touch (Echosense, Paris, France), which was performed using M or XL probe by an experienced gastroenterologist. The examination was defined as valid if there were ≥10 valid measurements with interquartile range-(IQR-) to-median ratio of LSM ≤0.3. The diagnosis of liver steatosis was considered in patients with CAP ≥ 238 dB/m [18]. On the contrary, patients with LSM ≥7 kPa were defined to have a significant liver fibrosis (≥F2), while an advanced fibrosis (≥F3) was considered if LSM was ≥9.6 kPa using the M probe or ≥9.3 kPa using the XL probe. Finally, patients with LSM ≥11.5 kPa using the M probe or ≥11.0 kPa using XL probe were defined as having cirrhosis. These cutoff values were taken from the earlier data [19, 20]. TE was done within a month of EGD.

2.5. Esophagogastroduodenoscopy. All included patients had EGD which was done by an experienced gastroenterologist with the help of EVIS EXERA III Gastroscope (Olympus, Tokyo, Japan). The diagnosis of GERD was made only on basis of EGD finding. Patients without endoscopic changes, but with GERD symptoms, were not characterized "as having GERD." The severity of GERD was defined according to the Los Angeles Classification. Hiatal hernia was defined if proximal dislocation of the gastroesophageal junction >2 cm above the diaphragmatic indentation [10, 21].

2.6. Statistical Analysis. Categorical variables are shown as percentages and continuous variables as means with standard deviation or medians with inter-quartile range. Difference between groups was tested using χ^2-test for categorical variables and *t*-test or Mann–Whitney where appropriate for continuous variables. Binary logistic regression was performed in order to identify parameters independently associated with occurrence of GERD. All statistical analyses were performed using SPSS V.27.0 (SPSS Inc, Chicago, IL, USA). Statistical tests were two-tailed and significance was set at 0.05.

3. Results

3.1. Demographic and Laboratory Characteristics of Study Subjects Divided by Controlled Attenuation Parameter (CAP) for Liver Steatosis. Demographic and laboratory characteristics of all 937 study subjects and the characteristics of the subjects classified according to CAP categories are listed in Table 1. The median age of the whole group was 49 (46–66) years. Women were more represented (54% vs. 46%). The mean BMI of the study population was $30.95 \pm 5.45\ \text{kg/m}^2$, while the mean WC was 105.51 ± 14.56 cm. The prevalence of NAFLD based on TE-CAP was 82.6% (774/937). In Table 1, there are patient characteristics with and without increased CAP. Briefly, those with increased CAP were younger and had higher BMI, WC, HC, and UAC, higher prevalence of hypertension, T2DM, and dyslipidemia. Regarding the laboratory examinations, patients with elevated CAP had higher values of liver test (AST and ALT), ferritin, serum uric acid, and fasting insulin. Similar results of higher prevalence in patients with elevated CAP were observed with GERD, hiatal hernia, and insufficient cardia. Additionally, patients with elevated CAP had higher prevalence of GERD grades B and C in comparison to those without elevated CAP. Consequently, patients who did not have elevated CAP had higher prevalence of GERD grade A.

3.2. Prevalence of Outcomes among Subjects Divided by Stages of Liver Fibrosis (Ranges F1 to F4). Even though we have found an upward trend in prevalence of GERD, hiatal hernia, and insufficient cardia, there was no significant difference between subjects with fibrosis (F)1-2 and F3-4 stage of fibrosis or F1 and F2-4 (defined by LSM). Prevalence according to the stage of liver fibrosis is shown in Table 2.

3.3. Association of GERD, Hiatal Hernia, Barrett's Esophagus, and Insufficient Cardia with Laboratory, Demographical Data and Elastographic Parameters: A Binary Logistic Regression. In a binary logistic regression, significant positive association with GERD was obtained for CAP. Results of this analysis are shown in Table 3. Furthermore, significant positive association with hiatal hernia was obtained for BMI, HDL cholesterol, and CAP (Table 4). Finally, significant positive association with hiatal hernia was obtained with CAP in multivariant analysis (Table 5). There was no association confirmed in multivariate analysis for Barrett's esophagus, due to the small number of patients with Barrett (Table 6).

4. Discussion

To the best of our knowledge, our study is the first to reveal a positive association between CAP as a surrogate marker of liver steatosis and GERD after adjustments for other clinical variables. Moreover, patients with elevated CAP had

Table 1: Demographic, laboratory, elastographic, and endoscopic characteristics of study subjects.

	All ($n = 937$)	Group 1 ($n = 163$), CAP < 238	Group 2 ($n = 774$), CAP ≥ 238	p value
Age, years (IQR)	49 (46–66)	53 (46–74)	47 (46–64)	0.007*
Gender				
Male, n (%)	431 (46)	67 (41)	364 (47)	0.190
Female, n (%)	506 (54)	96 (59)	410 (53)	0.190
Smokers				
Nonsmokers, n (%)	654 (69.80)	113 (69.33)	541 (69.89)	0.962
Active, n (%)	190 (20.28)	28 (17.18)	162 (20.93)	0.329
Ex, n (%)	93 (9.92)	22 (13.49)	71 (9.17)	0.125
Body height (cm)	169.53 ± 10.15	168.15 ± 9.94	169.81 ± 10.18	0.057
Body weight (kg)	89.10 ± 17.92	78.55 ± 18.22	91.28 ± 17.05	<0.001*
BMI (kg/m^2)	30.95 ± 5.45	27.64 ± 5.16	31.62 ± 5.27	<0.001*
Waist circumference (cm)	105.51 ± 14.56	96.94 ± 15.17	107.14 ± 13.88	<0.001*
Hip circumference (cm)	110.24 ± 12.39	104.15 ± 13.51	111.4 ± 11.83	<0.001*
Upper arm circumference (cm)	32.86 ± 6.6	30.28 ± 4.43	33.35 ± 6.85	<0.001*
Haemoglobin (g/L)	136.79 ± 18.01	129.51 ± 19.45	138.38 ± 17.29	<0.001*
Ferritin (ng/mL)	147.30 ± 152.24	124.27 ± 141.21	151.86 ± 154.04	0.035*
Thrombocytes	227.26 ± 64.79	225.73 ± 80.60	227.60 ± 60.90	0.738
Serum glucose (mmol/L)	7.34 ± 8.15	6.43 ± 4.04	7.57 ± 8.87	0.109
HbA1c (%)	7.18 ± 13.47	5.88 ± 1.2	7.46 ± 14.83	0.175
Serum uric acid (mmol/L)	352.80 ± 107.07	316.34 ± 115.72	359.55 ± 104.11	<0.001*
AST (U/L)	30.11 ± 26.42	24.49 ± 11.37	31.21 ± 28.33	0.003 *
ALT (U/L)	35.79 ± 26.98	26.70 ± 19.94	37.59 ± 27.82	<0.001*
ALP (U/L)	78.03 ± 39.16	82.20 ± 35.66	77.20 ± 39.80	0.138
GGT (U/L)	53.77 ± 58.66	48.39 ± 61.98	54.82 ± 57.98	0.204
Total cholesterol (mmol/L)	6.04 ± 9.82	4.82 ± 1.07	6.31 ± 10.81	0.079
HDL (mmol/L)	1.98 ± 8.67	1.56 ± 0.4	2.07 ± 9.51	0.494
LDL (mmol/L)	3.58 ± 8.67	2.74 ± 0.98	3.75 ± 9.53	0.177
Triglyceride (mmol/L)	2.34 ± 8.04	1.36 ± 1.80	2.54 ± 8.80	0.089
Albumin (g/L)	44.28 ± 7.73	44.14 ± 3.99	44.31 ± 8.33	0.799
CRP (mg/L)	5.56 ± 15.52	6.61 ± 17.87	5.34 ± 14.98	0.342
Serum insulin (pmol/L)	20.32 ± 24.51	12.03 ± 8.02	22.02 ± 26.34	<0.001*
Arterial hypertension, n (%)	629 (67.13)	93 (57.06)	536 (69.25)	0.004*
Diabetes mellitus, n (%)	339 (36.18)	41 (25.15)	298 (38.50)	0.002*
Hyperlipoproteinemia, n (%)	568 (60.62)	76 (46.63)	492 (63.57)	<0.001*
CAP (dB/m)	297.76 ± 61.56	198.28 ± 29.78	318.7 ± 43.33	<0.001*
LSM (kPa)	6.72 ± 4.08	5.13 ± 2.62	7.06 ± 4.25	<0.001*
GERD, n (%)	293 (31.27)	30 (12.88)	263 (33.98)	<0.001*
GERD grade A, n (%)	193 (20.59)	24 (80)	169 (64.25)	0.041*
GERD grade B, n (%)	48 (5.12)	2 (6.66)	46 (17.49)	0.013*
GERD grade C, n (%)	47 (5.01)	2 (6.66)	45 (17.11)	0.015*
GERD grade D, n (%)	5 (0.51)	2 (6.66)	3 (1.14)	0.182
Barrett's esophagus, n (%)	14 (1.49)	1 (0.61)	13 (1.6)	0.504
Hiatal hernia, n (%)	402 (42.9)	40 (24.54)	362 (46.77)	<0.001*
Insufficient cardia, n (%)	445 (47.49)	53 (32.52)	392 (50.65)	<0.001*

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; CAP: controlled attenuation parameter; LSM: liver stiffness measurement; GERD: gastroesophageal reflux disease. *$p < 0.05$.

significantly higher prevalence of higher GERD grades (B and C). Similar result was published by other authors [3, 6, 17]. For example, in the study by Hung WC et al. [17], which was published a few years ago, there was a positive association between NAFLD and erosive esophagitis independent of obesity. In their study, NAFLD was diagnosed based on abdominal ultrasound [17]. However, ultrasound is a good method for the detection of moderate-severe fatty liver, but the sensitivity of ultrasound decreases with the decrement of fatty infiltration, so in the presence of a hepatic fat content of 10% to 19%, it had a sensitivity of only 55% shown in a study on 100 living liver donor candidates [22, 23]. In our study, patients with elevated CAP were more obese; however, in a binary logistic regression, only elevated CAP values were associated positively with GERD. Thus, it is possible that elevated CAP (i.e., NAFLD) have a greater impact on the risk of GERD than obesity. Similar observation was reported by another earlier mentioned study [17] and by a recent meta-analysis [3]. Also, Fujikawa et al. [10] showed that severer GERD symptoms in NAFLD compared to the controls were observed independently of degree of BMI. On the other hand, some authors did not confirm the

Table 2: Prevalence of outcomes, gastroesophageal reflux disease, Barrett's esophagus, hiatal hernia, and insufficient cardia, among subjects divided by stages of liver fibrosis (ranges F1 to F4).

	F1 ($n = 663$)	F2 ($n = 133$)	F3 ($n = 36$)	F4 ($n = 105$)
GERD, *n* (%)	190 (28.66)	43 (32.33)	15 (41.67)	36 (34.29)
BE, *n* (%)	7 (1.06)	7 (1.50)	2 (5.56)	3 (2.86)
HH, *n* (%)	274 (41.33)	57 (42.86)	19 (52.78)	52 (49.52)
INSUF, *n* (%)	313 (47.21)	60 (45.11)	21 (58.33)	51 (48.57)
	F1-2 ($n = 796$)	F2-4 ($n = 274$)	F3-4 ($n = 141$)	
GERD, *n* (%)	233 (29.27)	94 (34.31)	51 (36.17)	
BE, *n* (%)	9 (1.13)	7 (2.55)	5 (3.55)	
HH, *n* (%)	331 (41.58)	128 (46.72)	71 (50.35)	
INSUF, *n* (%)	373 (46.86)	132 (48.18)	72 (51.06)	
	F1-2 vs. F3-4 *p* value	F1 vs. F2-4 *p* value		
GERD	0.123	0.102		
BE	0.071	0.102		
HH	0.065	0.149		
INSUF	0.407	0.843		

GERD: gastroesophageal reflux disease; BE: Barrett's esophagus; HH: hiatal hernia; INSUF: insufficient cardia.

Table 3: Association of GERD and laboratory, demographic data, and elastographic parameters.

	OR	95% CI	*p* value
BMI (kg/m^2)	1.00	0.95–1.05	0.955
Age (years)	1.01	0.99–1.03	0.543
AST (U/L)	1.00	0.99–1.01	0.730
ALT (U/L)	1.01	0.99–1.02	0.358
GGT (U/L)	1.00	0.99–1.00	0.606
Total cholesterol (mmol/L)	1.01	0.97–1.05	0.572
HDL (mmol/L)	0.76	0.40–1.45	0.407
LDL (mmol/L)	0.89	0.69–1.15	0.374
Triglyceride (mmol/L)	0.98	0.87–1.10	0.744
Serum insulin, pmol/L	1.00	0.99–1.01	0.873
Arterial hypertension, *n* (%)	1.33	0.76–2.34	0.319
Diabetes mellitus, *n* (%)	1.09	0.63–1.88	0.765
Hyperlipoproteinemia, *n* (%)	0.89	0.53–1.49	0.661
LSM (kPa)	1.00	0.95–1.06	0.964
CAP (dB/m)	1.01	1.01–1.02	<0.001*

BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; LSM: liver stiffness measurement; LDL: low-density lipoprotein; CAP: controlled attenuation parameter. *$p < 0.05$.

Table 4: Association of hiatal hernia and laboratory, demographic data, and elastographic parameters.

	OR	95% CI	*p* value
BMI (kg/m$^{2)}$	1.05	1.006–1.094	0.024*
Age (years)	1.00	0.983–1.020	0.894
AST (U/L)	1.01	0.990–1.026	0.396
ALT (U/L)	1.00	0.985–1.011	0.734
GGT (U/L)	1.00	0.993–1.002	0.224
Total cholesterol (mmol/L)	1.08	0.889–1.302	0.450
HDL (mmol/L)	1.38	1.053–1.799	0.019*
LDL (mmol/L)	0.76	0.566–1.023	0.070
Triglyceride (mmol/L)	0.91	0.785–1.058	0.222
Serum insulin (pmol/L)	1.00	0.990–1.007	0.699
Arterial hypertension, *n* (%)	1.14	0.689–1.895	0.605
Diabetes mellitus, *n* (%)	0.93	0.578–1.490	0.757
Hyperlipoproteinemia, *n* (%)	1.04	0.656–1.633	0.884
CAP (dB/m)	1.01	1.003–1.010	0.001*

BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CAP: controlled attenuation parameter. *$p < 0.05$.

connection among NAFLD and GERD [5]. However, further studies on this topic are needed.

Obesity is a well-known risk factor for GERD. We know from the earlier data that adipose tissue is metabolically active tissue that produces various inflammatory cytokines. Those cytokines relate to complications of GERD [24]. Other important factors that are involved in the pathogenesis of obesity and GERD are higher number of transient relaxations of the lower esophageal sphincter, the increased prevalence of esophageal motor disorders, and increased intra-abdominal pressure [24]. Our results showed that elevated CAP as a surrogate marker of liver steatosis (i.e., NAFLD) was associated with GERD. Our results raise the question of whether NAFLD can be involved in the pathogenesis of GERD. For over a century and a half, the important role of liver in the context of metabolism regulation has been recognized. However, fatty liver has for a long time been considered a trivial finding and just during the last 5–10 years the importance of NAFLD, not only for liver-related morbidity and mortality but as a condition that is connected to many extrahepatic diseases and cancers, has been recognized [25, 26]. NAFLD could be related to GERD via several mechanisms. Firstly, today we know from earlier data that in NAFLD patients there is an increased production of various proinflammatory cytokines, for example, interleukin-1 (IL-1), interleukin-6 (IL-6), TNF-alfa, TGF-beta, plasminogen activator inhibitor-1, increased reactive oxygen species, etc. These cytokines are produced by hepatocytes and non-parenchymal cells (Kupffer cells and hepatic stellate cells) [25–27]. It has been proposed that cytokines such as IL-1 and IL-6 could contribute to GERD development. Secondly, it is hypothesized that enhanced oxidative stress could lead to depletion of the adherent mucus layer and consequently damage esophageal mucosa. On the other hand, decreased antioxidant capacity is less

Table 5: Association of insufficient cardia and laboratory, demographic data, and elastographic parameters.

	OR	95% CI	p value
BMI (kg/m^2)	1.04	0.999–1.086	0.057
Age (years)	1.01	0.987–1.023	0.609
AST (U/L)	1.00	0.990–1.018	0.589
ALT (U/L)	1.00	0.986–1.009	0.672
GGT (U/L)	1.00	0.992–1000	0.068
Total cholesterol (mmol/L)	1.13	0.745–1.725	0.558
HDL (mmol/L)	1.17	0.901–1.519	0.238
LDL (mmol/L)	0.84	0.532–1.340	0.473
Triglyceride (mmol/L)	0.92	0.810–1.053	0.238
Serum insulin (pmol/L)	1.00	0.987–1.006	0.466
Arterial hypertension, n (%)	1.05	0.636–1.727	0.853
Diabetes mellitus, n (%)	0.89	0.555–1.418	0.617
Hyperlipoproteinemia, n (%)	1.01	0.640–1.584	0.977
CAP (dB/m)	1.01	1.003–1.011	0.001*

BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CAP: controlled attenuation parameter. *$p < 0.05$.

Table 6: Association of Barrett's esophagus and laboratory, demographic data, and elastographic parameters.

	OR	95% CI	p value
BMI (kg/m^2)	0.66	0.385–1.138	0.135
Age (years)	0.86	0.672–1.109	0.248
AST (U/L)	0.98	0.742–1.281	0.858
ALT (U/L)	1.02	0.877–1.191	0.783
GGT (U/L)	1.01	0.980–1.036	0.613
Total cholesterol (mmol/L)	0.97	0.716–1.323	0.861
HDL (mmol/L)	0.41	0.037–4.675	0.467
LDL (mmol/L)	4.69	0.591–37.216	0.144
Triglyceride (mmol/L)	0.56	0.081–3.859	0.556
Serum insulin (pmol/L)	1.03	0.951–1.111	0.486
Arterial hypertension, n (%)	1.00	—	0.994
Diabetes mellitus, n (%)	0.12	0.001–22.593	0.431
Hyperlipoproteinemia, n (%)	1.54	0.041–57.392	0.816
CAP (dB/m)	1.10	0.969–1.238	0.147

BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CAP: controlled attenuation parameter.

able to prevent damage of esophageal mucosa and because of that the severity of GERD is increased [17, 28, 29]. And in the context of NAFLD there is increased systemic oxidative stress and a lower antioxidant capacity [17]. Thirdly, earlier data have shown that triglyceride could affect the lower esophageal sphincters. Hypertriglyceridemia is strongly associated with NAFLD and some authors have reported that this could be the shared underlying factor between GERD and NAFLD [30–33]. In our study, patients with elevated CAP had higher prevalence of dyslipidemia; however, we did not find serum triglyceride to be associated with GERD. But regarding the fact that patients were taking statins, we did not expect to find that association. Fourthly, autonomic nervous system dysfunction could represent additional link among NAFLD and GERB, because some data have reported that in NAFLD patients there is a higher prevalence of autonomic disturbance. On the other hand, it has been reported that autonomic dysfunction could be responsible for the abnormal gastric and esophageal motility and consequently it may predispose to development of GERD [3, 34, 35]. Finally, NAFLD is associated with obesity, especially with central obesity. We know that visceral fat is a metabolic active tissue responsible for releasing of proinflammatory cytokines. Also, regarding the fact that most of NAFLD patients are obese, there is direct mechanical effect on increasing gastric pressure which for the consequence has often lower esophageal sphincter relaxation with reflux of gastric acid [7, 17, 36]. Thus, enhanced oxidative stress and subchronic inflammatory state with release of inflammatory cytokines in the NAFLD patients, as well as strong correlation of NAFLD with central obesity, connect NAFLD with GERD development [17, 25, 26]. But further studies that will better investigate this association are needed.

Furthermore, the presence of insufficient cardia and hiatal hernia is associated with GERD [24]. In our study, obesity (defined by BMI) and CAP were independent predictors of presence of hiatal hernia. These results are in line with the connection of NAFLD and obesity. Recently, we have shown that CAP as a surrogate marker of NAFLD is correlated with MetS, obesity, and other MetS components [37, 38].

Finally, we had investigated the relationship of LSM as a surrogate marker of liver fibrosis with the presence insufficient cardia, hiatal hernia, GERD, and BE. Although there was no significant difference between subjects F1-2 and F3-4 stage of fibrosis or F1 and F2, we have found an upward trend in prevalence of GERD, hiatal hernia, and insufficient cardia according to the stage of liver fibrosis. We believe that with a larger number of patients this could reach a statistical significance. This data is in accordance with earlier observation that NAFLD is a multisystem disease and that degree of fibrosis is the strongest factor related to extrahepatic diseases that relate to NAFLD [14]. Further investigations on this topic are needed.

Nevertheless, our study has some limitations. Firstly, the cross-sectional design of the study precludes any causal inferences about the directionality of the connections that were found in our study. By the design of our study, we cannot exclude that apparent association among CAP (i.e., NAFLD) and GERD may not be causal but is a result of shared underlying risk factors (i.e., metabolic risk factors). Secondly, we did not use LB for NAFLD diagnosis. However, LB is an invasive procedure, and it would be non-ethical to perform LB in these patients. We used one of the best and widely available non-invasive methods that was reported as a good method for noninvasive assessment of liver steatosis and fibrosis [16]. According to our best knowledge, this is the first study in Croatia, in this part of Europe, which investigated the association among NAFLD and GERD. Thus, we have analyzed our single-center experience and our cohort should not be considered strictly representative of the general population. Furthermore, earlier data reported negative association among GERB and *Helicobacter pylori* infections [39]. In our cohort, we did not have information regarding this infection. There was no association confirmed

in multivariate analysis for Barrett's esophagus, due to the small number of patients with BE; thus, further and larger studies are needed. However, our study was the first to date that investigated the association among elastographic parameters of liver steatosis and fibrosis (i.e., CAP and LSM) and GERD. It has the strength of a relatively large sample size and we use CAP and LSM obtained by TE that are one of the best validated non-invasive methods for the assessment of liver steatosis and fibrosis. Moreover, CAP and LSM measurements were assessed by using both FibroScan probes (M and XL). Finally, GERD was defined by "gold standard," i.e., esophagogastroduodenoscopy.

In conclusion, our results demonstrate a significant association among CAP and GERD. Thus, in everyday clinical practice, we should pay more attention to NAFLD patients as they probably have an increased GERD risk. Further, longitudinal studies that will investigate this association and that will help us to understand underlying mechanisms between NAFLD and GERD are needed. Also, further studies could answer the question of whether by the use of noninvasive method (CAP and LSM) we could recognize those with GERD, especially those with severe forms that should undergo upper gastrointestinal endoscopy in order to prevent GERD complications, i.e., BE and esophageal cancer. This is important regarding the fact that, with the increase in incidence of obesity and MetS, the incidence of NAFLD is also increasing, and consequently, we can expect an increase in GERD incidence as well.

Data Availability

Access to data is restricted due to ethical restrictions.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

All authors contributed equally to this review. All authors read and agreed on the published version of the manuscript.

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Review Article

NAFLD, Insulin Resistance, and Diabetes Mellitus Type 2

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Nonalcoholic fatty liver disease is a condition defined by fat accumulation in hepatocytes not promoted by excessive alcohol consumption. It is highly prevalent and is strongly associated with insulin resistance, metabolic syndrome, and diabetes type II. Insulin resistance plays a crucial role in the multifactorial etiopathogenesis of this condition leading to accumulation of free fatty acids in the liver cells, thus causing lipotoxicity, inflammation, and fibrosis. In this review, we will focus on currently known pathogenesis of nonalcoholic fatty liver disease. Numerous investigation strategies are available to establish the diagnosis, from biochemical markers and ultrasound to various molecular and advanced imaging techniques and liver biopsy. Prevention is crucial. However, effective and promising therapies are strongly demanded.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined as fat accumulation in the liver cells in patients without excessive alcohol consumption. To confirm the diagnosis, more than 5% of hepatocytes must contain lipid droplets when analyzed on light microscopy [1]. NAFLD can be further divided into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) depending on whether or not inflammation is present. Although it was historically, and perhaps even today in some cases, considered as benign, this condition must be taken seriously as it can lead to liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and liver failure. What is worrying is that we see significant rise in prevalence not only in NAFLD but as well in other conditions accompanying this disease [2]. This has led NAFLD to become the most prevalent liver disorder in the last few decades [3]. According to recent statistics, there will be even greater rise in liver cirrhosis and other sequels of liver steatosis and steatohepatitis. At the moment, NAFLD is the second cause of liver disease in patients requiring liver transplantation in the USA and is expected to become number one cause for liver transplantation [4]. There is plethora of evidence that NAFLD does not only affect the liver but also associate with metabolic syndrome (MetS), type II diabetes (T2D), as well as with cardiovascular disease and chronic kidney disease (CKD). NAFLD and T2D have similar risk factors, and epidemiology and pathophysiology further emphasize their connections [5]. Evidence show that NAFLD is associated with one or more of the MetS components—arterial hypertension, central obesity, dyslipidemia, insulin resistance (IR), and T2D. The more components are present, higher are the chances for NAFLD and eventually advanced fibrosis [6]. Global NAFLD prevalence in T2D, according to meta-analysis including almost 50 thousand patients from 80 studies, was found to be as high as 55.5%. Other research showed a prevalence of NAFLD in T2D up to 59.67% and even 77.87% in obese T2D patients [7]. Remarkable, up to 5-fold increase in risk for developing T2D in patients with NAFLD was observed [8]. Once considered the hepatic manifestation of MetS, NAFLD in modern terms represents continuum from obesity to MetS and T2D [9] as there is a growing number of data suggesting that it can precede to these conditions [10]. But whether or

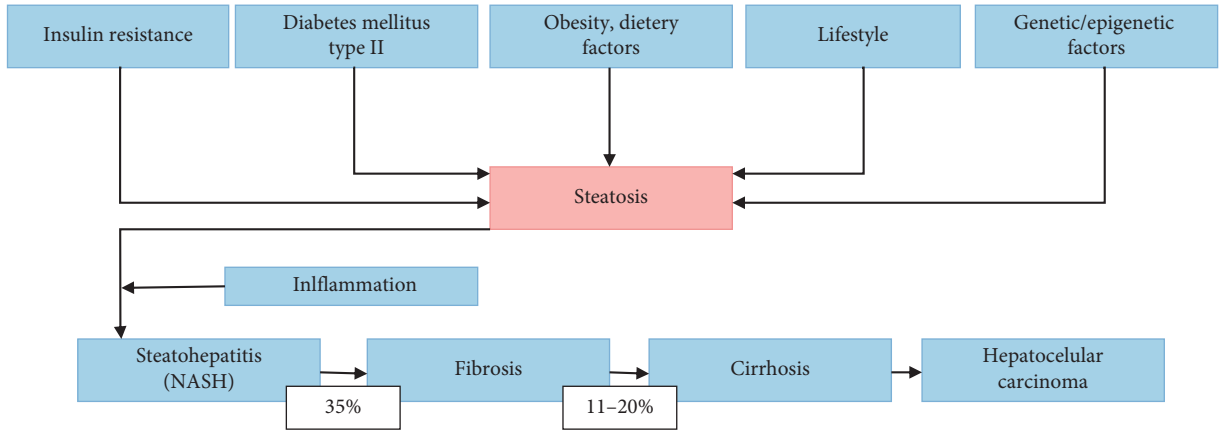

Figure 1: Progression of steatosis.

not NAFLD is a preceding state to MetS and T2D or their consequence, it is clear that there is a vast spectrum of different signaling molecules which all interact on different levels and start a vicious self-perpetuating circle, making it hard to say what is the first "hit." In this review, we will give comprehensive summary of pathogenesis of NAFLD focusing on insulin resistance, MetS, and T2D and their interaction as they play the central role of liver steatosis.

2. Pathogenesis

Pathogenesis of NAFLD is still incompletely understood as there is more than one factor contributing to this condition. Dysregulation of lipid delivery, hepatic lipid uptake, oxidation, synthesis, and secretion in very low density lipids promotes steatosis. Not all patients with liver steatosis will develop steatohepatitis, and this was initially explained by the two hit theory [11]. Certain lifestyles, combining lack of physical activity, high fat diet, and obesity, were recognized to cause steatosis as the first hit. If second hit was to occur, then it would trigger inflammation and fibrosis. Recent evidence claim the two hit hypothesis obsolete as it cannot explain multiple insults acting together on different metabolic and molecular levels [12]. Insulin resistance is just one of them, potentially most important, among other factors including adipokines such as leptin, adiponectin, resistin, gut microbiota, and other genetic, epigenetic, and environmental factors. Progression of steatosis can be seen in Figure 1.

2.1. Insulin Resistance. Insulin resistance plays pivotal role in liver steatosis and even more so in steatohepatitis. The term was first used almost one century ago, after the introduction of insulin therapy. Due to the low quality of the first insulin which caused production of antibodies, high doses of insulin were required, leading to overtreatment/exogenous hyperinsulinemia. Insulin resistance was defined as "a state in which a greater than normal amount of insulin is required to elicit a quantitatively normal response" [13]. Several decades later, hyperinsulinemia was recognized as an endogenous pathophysiologic mechanism, raising from insulin resistance and leading to metabolic and endocrine disruptions [14]. Today, we know that IR plays a crucial role in impaired glucose homeostasis, MetS, and T2D.

Insulin binds to the insulin receptor (a tetramer consisting of two alpha and two beta chains) on the cell surface. When insulin binds to the alpha chain, it activates a signaling cascade subsequently promoting glucose transport (glucose influx), glycogen synthesis, lipogenesis, and cell proliferation, differentiation, and survival. One the other hand, this cascade leads to downregulation of gluconeogenesis and lipolysis. The cellular insulin signaling pathway is a complex process consisting of several steps. Everything starts with autophosphorylation of the insulin receptor beta chain which activates the insulin receptor substrate (IRS 1/2). IRS activation then triggers three main pathways: PI3K/AKT (responsible for the metabolic insulin action via the translocation of the glucose transporter type 4 (GLUT4) to the plasma membrane), TSC1/2-mTOR (playing a critical role in protein synthesis since target of mTOR is a central controller for processes including RNA translation, ribosome biogenesis and autophagy, in response not only to growth factors and hormones like insulin but also to nutrients, energy, and stress signals), and RAS-MAPK pathway (promoting cell survival, division, and motility via extracellular signal-regulated kinase 1/2 (ERK1/2) complex that translocates into the nucleus activating many transcription factors, constituting an important connection between the cytoplasmic and nuclear events and orchestrating gene expression, mitogenesis, and differentiation) [15–17]. Despite greater understanding of molecular pathways in insulin signaling and metabolism, there are still numerous knowledge gaps regarding the etiology of IR in several metabolic disturbances such as NAFLD where insulin resistance seems to play crucial role.

Alterations in any of the steps in insulin signaling cascade can lead to IR, which is seen on the cellular level due to dysregulation of intracellular signals normally promoted with insulin binding. Different types of kinases and phosphatases are responsible for balancing this signaling cascade. Generally, tyrosine phosphorylation activates and serine/threonin phosphorylation inactivates insulin receptor and IRS proteins [18]. In IR, this process is

mediated by several enzymes including inhibitor of kappa kinase beta (IKK-b), c-Jun-N-terminal kinase (JNK-1), and protein kinase C (PKC) which all promote serine phosphorylation of IRS and thus decrease glucose uptake, glycogen synthase activation, and also phosphorylation of forkhead box protein O (FOXO) which then result in hepatic gluconeogenesis stimulation [19, 20]. FFA, oxidative stress, and adipocyte mediating alterations are main causes of the aforementioned IKK-b, JNK-1, and PKC influences on the inhibition of IRS 1/2 signaling. Investigations also showed that inflammatory cytokines such as TNF-alpha, IL-1 beta, and IL-6 can induce serine phosphorylation of IRS1 through JNK-1, IKKb, S6K, and mTOR and induce insulin resistance [21–24]. Adipose tissue additionally plays an important role in IR as it is highly metabolic active secreting adipokines such as leptin, resistin and adiponectin. Leptin has a significant effect on IRS 1 dephosphorylation, but it is believed its role is mediated by the central nervous system rather than peripherally [25, 26]. Furthermore, leptin has a significant impact on liver fibrosis via transforming growth factor beta 1 [27]. Adiponectin levels were shown to correlate positively with insulin sensitivity, but on the other hand, it has a negative impact on inflammatory markers and TNF-alpha which induces IR, so low levels of adiponectine could potentially be significant factor of IR and lead to NAFLD [28]. Not only disruption of initiation of insulin signaling formerly explained but also termination of signaling cascade has an important role in IR. There are two most important phosphatases which terminate insulin signaling, phosphatase and tensin homolog (PTEN), and Src homology 2 domain containing inositol 5′-phosphatase 2 (SHIP2) [29]. Their increased activity terminates insulin action. The mechanism of insulin resistance is not limited to impaired insulin signaling, but it also involves the complex interplay of multiple metabolic pathways. Recent analysis of large datasets generated by metabolomics and lipidomics has revealed the role of metabolites such as lipids (saturated and unsaturated fatty acids, branched fatty acid esters of hydroxy fatty acids, diacylglycerol, sphingolipids, ceramides, and phospholipids), amino acids (methionine, circulating aromatic amino acids (AAAs) such as phenylalanine and tryptophan, branched-chain amino acids such as leucine, isoleucine, and valine), ketone bodies, and bile acids in modulating insulin sensitivity. Metabolites can regulate insulin sensitivity directly by modulating components of the insulin signaling pathway, such as insulin receptor substrates (IRSs) and AKT, and indirectly by altering the flux of substrates through multiple metabolic pathways, including lipogenesis, lipid oxidation, protein synthesis and degradation, and hepatic gluconeogenesis [30]. The aforementioned are only a part of insulin resistance etiopathology, numerous other molecular pathways in addition play an important role, and more are to be discovered in future work.

So far, it seems that excess of free fatty acids (FFA) and hyperinsulinemia are essential to start the vicious self-perpetuating circle of NAFLD. Excess FFA are in part due to increased caloric intake and obesity as well as adipocyte resistance to insulin leading to lypolysis and hyperinsulinemia. This was shown to promote lipogenesis via sterol regulatory element binding protein (SREBP1-c). SREBP1-c is just one of the lipogenesis controlling factors among others, including carbohydrate response element-binding protein (ChREBP) [31] and X-box binding protein (XBP1) otherwise known as unfolded protein response regulator (UPR). Besides controlling lipogenesis, XBP1 regulates leptin resistance, adipogenesis, inflammation, and insulin signaling and is heavily affected by endoplasmatic reticulum stress. High serum FFA, high serum cholesterol, increased lypolysis due to IR, and de novo lipogenes, decreased very low density lipoproteins (VLDL) assembly [32] all leading to high levels of liver FFA. Subsequently, this leads to lipotoxicity of accumulated fatty acids through mitochondrial dysfunction and oxidative stress as well as endoplasmatic reticulum stress. FFA normally undergo beta oxidation in the mitochondria and peroxisome as well as omega oxidation in the microsomal system. It leads not only to energy production in the form of adenosine triphosphate but also to a production of small quantities of free radicals resulting in oxidative stress [33]. Oxidative stress has been linked to the production of highly reactive intermediates during inflammation. On the other hand, reactive oxygen species (ROS) are able to further enhance the inflammatory response by triggering proinflammatory mediators (e.g., NF-kB) and cytokine production (e.g., IL-6, IL-1, IL-1β, IL-18, resistin, lipocalin, and TNF-alpha). The consequences of this are very dangerous, especially for nucleic acids, where modification of bases, covalent crosslinks, and single- and double-strand breaks can occur. In addition to the radical species deriving from oxygen, other radicals are derived from reactive nitrogen species (RNS), e.g., the superoxide anion (O2−) [34]. Reactive oxygen and nitrogen species (ROS) cause damage in the cell nucleus and in the mitochondria. So, in a state of IR, there is an increase in beta oxidation and thus higher ROS production. Proinflammatory factors and adipokines are relevant not only in inducing IR but also in progression of steatosis to steatohepatis. We already described how TNF-alpha has an impact on IRS1 causing IR, but, on top of that, it recruits inflammatory cells to the liver and increases reactive oxygen stress through mitochondria and promotes cell death [35]. TNF-alpha has a negative effect on adiponectin further decreasing its protective role [36]. Besides lipotoxicity, oxidative stress it causes, and adipokines dysregulation, it appears that factors such as intestinal microbiota can also play pro-inflammatory role in NAFLD and development of insulin resistance [37]. Normally, it has a role in keeping the mucosa integrity by tight junctions and has an immunomodulatory effect on innate immunity. Dysbiosis can cause increased gut permeability, and endotoxemia can occur as lipopolisaharids and can enter portal circulation and aggravate innate immune response [38]. This effect can be explained by LPS impact on toll-like receptors 4 (TRL4) and signaling pathways resulting in TNF-alpha and interleukin-1-beta (IL-1b) release [39]. Production of short chain fatty acids (SCFAs) by gut microbiota is also involved in inflammation seen by disarranged diversity and amount of SCAFs in NAFLD [40, 41].

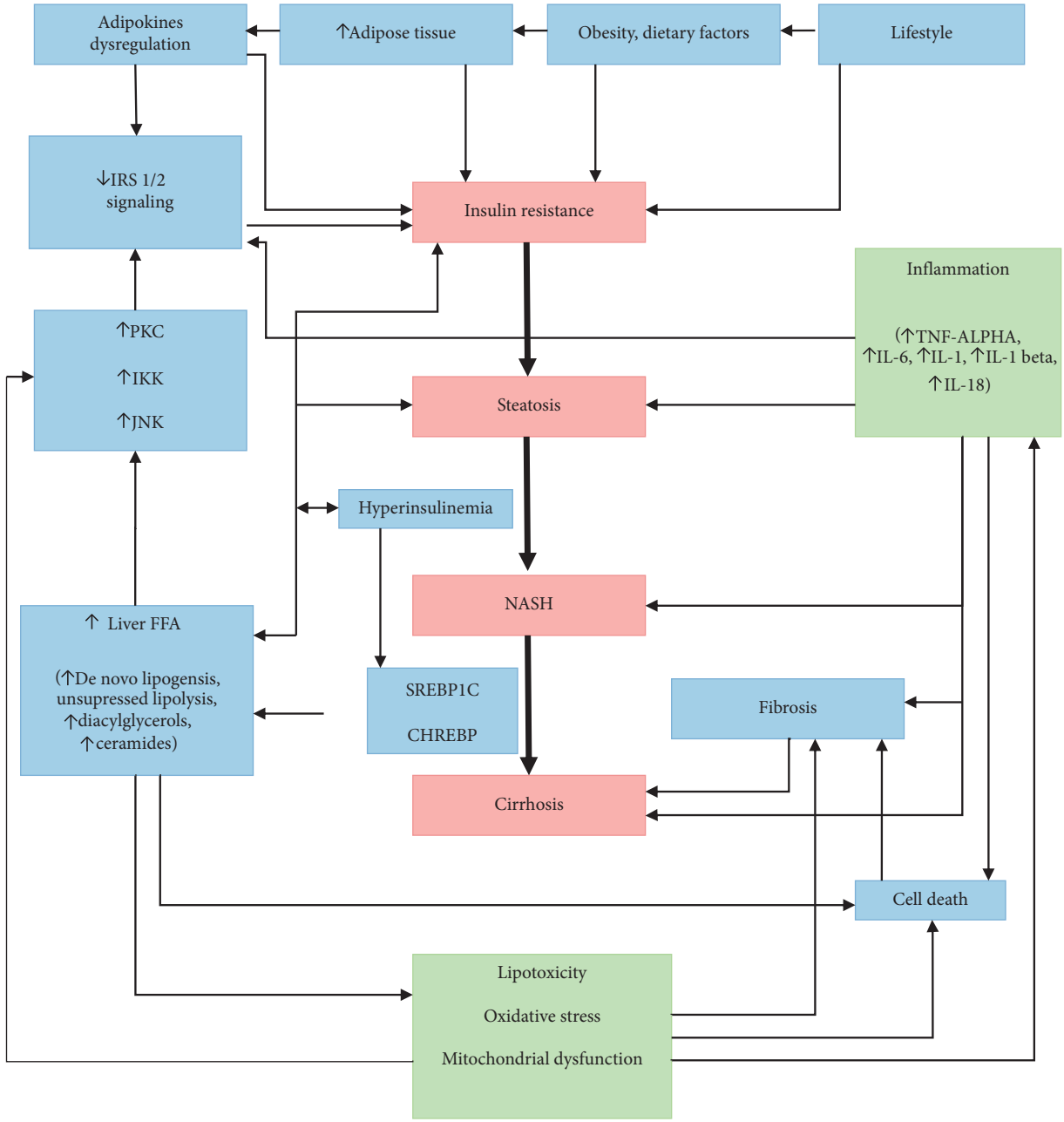

Figure 2: Pathophysiology.

Ultimately, lipotoxicity, inflammation, adipokine, and microbiome dysregulations will result in two main outcomes, and those are cell death and fibrosis. Hepatocyte cell death is the main trigger of progression of the disease. There are several different types of hepatocyte death, be that apoptosis, necrosis, pyroptosis, and necroptosis, but all will lead to inflammation and fibrogenesis [42]. Stellate cells are crucial in response to chronic liver injury. Activated stellate cells start to produce extracellular matrix proteins, dominantly collagen desposition. Collagen component can increase up to ten times in cirrhosis [43]. Stellate cells are not only important in fibrogenesis but also they have an inflammatory role as activated stellate cells are prone to LPS activation on the TLR4 pathway stimulating further cytokine release and activation of NK-kB and JNK pathways. All of the mechanisms we mentioned must be seen as an interactive complex happening parallel with each other (Figure 2). If the vicious circle is not stopped early, it will, in some patients, lead to cirrhosis, hepatocellular carcinoma, and liver failure as the end result of this condition. Such significant sequels will develop in up to 11–20% of NASH patients [44].

3. Diagnosis

NAFLD is usually discovered incidentally, by verifying elevated liver biochemical tests levels or as an incidental finding of hepatic steatosis using imaging methods. Most

patients are asymptomatic (48%–100%), but some have right upper quadrant pain, fatigue, or malaise. Hepatomegaly is often seen but difficult to differentiate on physical examination because of obesity. Typical changes for chronic liver disease such as splenomegaly, spider telangiectasia, palmar erythema, and ascites are seen in patients with NASH cirrhosis. To establish the diagnosis of NAFLD, alcohol-related liver disease must be excluded, which means consumption of less than 20–40 grams of alcohol per day.

In metabolic fatty liver disease, mild to moderate elevations of serum AST or ALT level or both are recorded, usually 2- to 4-fold elevations with AST/ALT ratio <1 in most patients. The serum alkaline phosphatase level is slightly elevated in one-third of patients as well as GGT, but the serum bilirubin, serum albumin level, and prothrombin time are normal, except in patients with NAFLD-associated cirrhosis. One-fourth of patients may have ANA in low titers (<1 : 320), but other laboratory tests for other chronic liver diseases are negative. Serum and hepatic iron levels may be elevated in 20%–50% of patients with NAFLD and may be a marker of more advanced disease. A serum ferritin greater than 1.5 times the upper limit of normal has been associated with higher NAS (NAFLD Activity Score) in a study of 628 adult patients with NAFLD [45]. Clinical and laboratory findings do not correlate with the histologic severity of NAFLD, and the entire histologic spectrum of NAFLD, including cirrhosis, can be seen in patients with normal or near normal serum aminotransferase levels [46].

Imaging techniques are obtained for the evaluation of unexplained liver biochemical test abnormalities or suspected NAFLD. Ultrasound may show a "bright," hyperechogenic liver, consistent with liver steatosis, and fatty liver can also be seen on abdominal CT or by MRI, but all these imaging methods cannot confirm the presence or determine the severity of NASH.

4. When to Perform Biopsy?

The reality is that most patients with NAFLD, diagnosed when hepatic steatosis is present on cross-sectional imaging studies and other chronic liver diseases are excluded, do not undergo a liver biopsy although it is required to identify patients with NASH. Liver biopsy is an invasive procedure with rare but severe complications, but it is important to differentiate patients with NASH because they are at risk of progression to cirrhosis or even HCC. That is why advanced imaging, laboratory tests, and scoring systems have been introduced to identify high-risk patients who should undergo liver biopsy.

Advanced imaging techniques include US-based technology of vibrations-controlled transient elastography (VCTE or FibroScan) which uses a low-amplitude shear wave that propagates through the liver parenchyma and magnetic resonance elastography (MRE) which combines MRI with elastography. A prospective work from Siddiqui et al. demonstrated that VCTE accurately distinguishes low from advanced stages of fibrosis but is less accurate in distinguishing intermediate stages of fibrosis or the presence of NASH [47]. MRE is excellent for staging liver fibrosis and is superior to VCTE but at higher cost and limited availability because specific MRI software and hardware are required.

Noninvasive laboratory tests have been developed to estimate the presence of steatohepatitis or fibrosis. One of them, the most promising single marker for identifying NASH is cytokeratin 18 (CK-18), a marker of apoptosis, but does it have enough sensitivity and specificity to be used alone as a predictive marker for NASH is still unknown [48, 49].

Various clinical scoring systems have also been analyzed for their ability to predict NASH or advanced fibrosis. The major clinical scoring systems include FibroTest, FibroMeter, NAFLD fibrosis score, Fibrosis-4, AST-to-platelet ratio (APRI), BARD (BMI, AST/ALT ratio, and diabetes mellitus), Enhanced Liver Fibrosis (ELF) score, NashTest, and AST/ALT ratio. Comparison of these tests in terms of positive and negative predictive values generally has demonstrated that more complicated and expensive tests are not more accurate than basic laboratory tests. These tests are good at predicting absence or advanced fibrosis and are not helpful in distinguishing intermediate stages of fibrosis [50]. NAFLD fibrosis score is the most commonly used clinical scoring algorithm that incorporates age, BMI, hyperglycaemia, AST/ALT ratio, platelet count, and serum albumin level. A low cutoff value for this score has been shown to have a high negative predictive value of 88%–93%, and a high cutoff value has shown a good positive predictive value of 82%–90%. This leaves 1 in 4 patients as having intermediate result, and for this group, a liver biopsy would be required for accurate staging.

5. Treatment

Currently, there is no established treatment for NAFLD or NASH. Weight loss and low-fat diet are generally recommended. There is no consensus on the most effective pharmacological agents for the treatment of NAFLD and NASH because their multifactorial pathologies are not fully understood. Histologic improvement in steatosis, inflammation, and fibrosis is the ultimate goal of treatment. Treatment strategies are now grouped into lifestyle modification, surgical interventions for weight loss, and pharmacotherapy.

5.1. Lifestyle Modification. Lifestyle modification includes reduction in energy intake and increase in physical activity with final goal of weight loss. Weight loss has been demonstrated to reduce liver transaminases [51–53] and decrease liver fat content. Several randomized controlled trials have shown an improvement in hepatic histology after calories intake restrictions leading to weight loss. One large prospective trial of 261 patients followed for 12 months demonstrated that all features of NASH improved with weight loss of at least 10% and fibrosis stabilized or improved with weight loss of at least 5% [54]. It has been reported that Mediterranean diet is an effective nonpharmaceutical option

for diabetes type 2 and obesity [55, 56] and may improve hepatic steatosis [57], but there is no evidence that Mediterranean diet alone, without general reduction of caloric intake can be beneficial. Silymarin, the extract of milk thistle, has been used for the prevention of liver fibrosis by regulating the antifibrogenic and anti-inflammatory functions [58] and is associated with the reduction of insulin resistance and improvement in liver function [59, 60].

Omega-3 fatty acids are approved in USA for hypertriglyceridemia and have been discussed as a potential treatment for NAFLD. A meta-analysis including 355 patients demonstrated the omega-3 supplementation improved hepatic steatosis, but no histologic data were available [61]. Other research failed to show benefits, so further work is required.

5.2. Bariatric Surgery. Bariatric surgery is not recommended as a treatment for NAFLD and NASH, but patients who underwent bariatric surgery for other reasons had a significant weight loss that resulted in improved metabolic parameters and hepatic histology. In was reported that 85% of patients with NASH who underwent bariatric surgery had resolution of NASH and 33% had improvement in fibrosis [62] on liver biopsies one year after bariatric surgery.

5.3. Pharmacotherapy. Numerous drugs have been investigated for the treatment of NAFLD, and they can be grouped in weight loss medications, insulin sensitizers, antioxidants, and cytoprotective or antifibrotic agents.

5.4. Weight Loss Medications. The most investigated medication is orlistat, a reversible inhibitor of pancreatic and gastric lipase. It promotes weight loss through intestinal fat malabsorption. Initial trails where promising, but in the end, there was no significant weight loss between the orlistat group and placebo group [63, 64]. Side-effects, such as oily stools and potential malabsorption of other medications and reports of cholelithiasis, cholestasis, and hepatic injury, have limited the benefits of this medication.

5.5. Diabetic Medications. Metformin, thiazolidinediones, and incretin mimetics have been studied in the treatment of NASH. Metformin reduces plasma glucose levels primarily by reducing hepatic glucose production through the activation of AMP (adenosine monophosphate-activated protein) kinase. Activation of this enzyme also results in decreased lipid synthesis and increased fat oxidation [65]. Results have been good in mice, where metformin reduced hyperinsulinemia and improved hepatic insulin sensitivity and reduced hepatomegaly and hepatic steatosis [66], but this effect was not observed in human studies [67, 68]. Currently, metformin is not recommended for treating NAFL and NASH.

Thiazolidinediones are peroxisome proliferator-activated receptor- (PPAR-) γ agonists, a nuclear receptor that is expressed in adipose tissue, muscle, and liver. Rosiglitazone and pioglitazone have shown to improve insulin resistance, normalization of liver biochemical test levels, and histologic improvements [69, 70]. Meta-analysis of Musso et al. has also confirmed reducing hepatic fibrosis in patients with NASH with or without diabetes mellitus [71].

Incretin mimetics-glucaon-like-protein-1 receptor agonists (GLP-1Ras) have been shown to reduce liver inflammation and fibrosis. Furthermore, glucagon receptor agonism is being investigated for the treatment of NAFLD due to its appetite-reducing effects, as well as its ability to increase lipid oxidation and thermogenesis. Recent data suggest that glucagon receptor signaling is disrupted in NAFLD, indicating that supraphysiological glucagon receptor agonism might represent a new NAFLD treatment target. Currently available GLP-1RAs which improve insulin sensitivity and serum glucose levels promote modest weight loss and lower hepatic transaminases are exenatide, dulaglutide, semaglutide, and liraglutide [72–74]. A randomized controlled trial of 52 patients where liraglutide was compared to placebo showed significant resolution in NASH in 39% patients treated with liraglutide compared to 9% treated with placebo [75].

5.6. Antioxidants. Vitamin E is a potent antioxidant. It is well tolerated, improves serum aminotransferase levels, reduces hepatic steatosis, and in nondiabetics, improves steatohepatitis but not fibrosis [76, 77]. Due to cardiovascular risks in diabetic patients, vitamin E is not recommended in diabetic patients with NAFLD [78].

Carotenoids are as potent as vitamin E in inhibiting lipid peroxidation [79], but carotenoid supplementation (β-cryptoxanthin and astaxanthin) has not been widely used as antioxidant treatment for patients with NASH.

6. Conclusion

NAFLD is not an isolated condition, but a fragment of metabolic disruption emerging from high energy intake, obesity, sedentary lifestyle, and crucially IR and T2D. Prevalence of NAFLD and T2D is in remarkable rise, as both have similar risk factors, epidemiology, and pathophysiology. The presence of T2D significantly increases the chances of developing NASH and fibrosis compared to NAFLD without T2D. Relation between NAFLD and T2D is not as straightforward and these conditions have multiple interactions on different molecular levels we tried to summarize in our text. Evidence suggests that NAFLD can precede T2D, so, perhaps, by effectively managing NAFLD, we could modify the risk for T2D development in the future. Although NAFLD will eventually, in some patients, progress to liver cirrhosis and hepatocellular carcinoma, these are not the main outcomes of NAFLD as just a proportion of NASH patients will develop such significant sequels. What is more notable is the cardiovascular risk, these patients have, and cardiovascular disease are the main causes of mortality in NAFLD which further emphasizes the metabolic component of this condition. That is why screening for T2D and MetS is important when we encounter with NAFLD patients in everyday practices. The same should be done in treating

patients with T2D-attention must be given to eventual concomitant liver manifestations. Unfortunately, there is no simple method in treating NAFLD and NASH, so prevention is of crucial importance. Promising multifunctional therapies are much awaited.

Data Availability

The data used to support this study are included within article as references.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

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Research Article

Assessment of Steatosis and Fibrosis in Liver Transplant Recipients Using Controlled Attenuation Parameter and Liver Stiffness Measurements

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Aim. The primary objective of this study was to evaluate the prevalence of increased controlled attenuation parameter (CAP) and liver stiffness measurements (LSM) as surrogate markers of liver steatosis and fibrosis in liver transplant recipient (LTR). Secondary objectives were to determine the predictors of increased CAP and LSM in population of LTR. *Methods*. In this prospective, cross-sectional study, we have evaluated 175 LTRs' mean age as 61 (53–65) with a functioning graft for more than one year who came for regular outpatient examinations to the Department of Gastroenterology, University Hospital (UH) Merkur, Zagreb, Croatia. *Results*. Of 175 analyzed LTRs, 34.28% had obesity, 64.00% had hypertension, 38.28% had diabetes, and 58.85% had hyperlipidemia. The prevalence of liver steatosis was 68.57%, while the prevalence of severe liver steatosis was 46.85%. On multivariate analysis, independent factors associated with liver steatosis were male gender, total cholesterol as positive predictor, and HDL as negative predictor, and independent factors positively associated with severe liver steatosis were higher body mass index (BMI) and higher triglyceride levels. The prevalence of moderate liver fibrosis was 54.85%, while the prevalence of advanced liver fibrosis was 24%. On multivariate analysis, independent factors positively associated with moderate fibrosis were gamma-glutamyl transferase (GGT) and CAP, while the independent factor positively associated with advanced fibrosis was GGT. *Conclusion*. Our study showed high prevalence of increased CAP and LSM measurements as surrogate markers of liver steatosis and fibrosis. Metabolic syndrome components were highly present and were associated with CAP and LSM values as well as in the pretransplant setting. Due to high prevalence of metabolic comorbidities and nonalcoholic fatty liver disease in LTRs and the lack of the abnormal liver test in a significant number of these patients, TE with CAP may be a reasonable initial assessment for LTRs with one or more components of the metabolic syndrome.

1. Introduction

The prevalence of obesity, diabetes mellitus type 2, and metabolic syndrome (MetS) is increasing; therefore, non-alcoholic fatty liver disease (NAFLD) is becoming the most important chronic liver disease (CLD) today. According to the data, NAFLD affects around 25% of the total population. NAFLD is a liver manifestation of MetS and is in close relationship with MetS and its individual components (i.e., diabetes mellitus type 2, obesity, dyslipidemia, and hypertension). Today, we evaluate NAFLD as a multisystem disease because in the past ten years, a large amount of data had connected NAFLD with numerous extrahepatic chronic diseases such as cardiovascular diseases (CVD), chronic kidney disease (CKD), and type 2 diabetes mellitus. [1]. A subset of NAFLD patients will develop end-stage liver disease (ESLD) (i.e., cirrhosis and/or hepatocellular carcinoma (HCC)) [1–7]. Additionally, emerging results suggest that HCC can evolve even in noncirrhotic NAFLD [3]. Fatty liver disease is of great interest for many authors who manage patients with liver transplant because it has multiple impacts in the context of liver transplantation (LT) [3, 8]. For the first, NAFLD-related ESLD (i.e., cirrhosis and HCC) has become one of the leading indications for LT in the USA. It is expected that NAFLD will become the leading indication for LT in the next 20 years due to epidemic raise in the incidence of MetS and its individual components [3, 8, 9]. Second, the challenging issue in the context of NAFLD and LT is also a liver allograft steatosis which is in direct relationship with a pool of potential donors. Because of epidemic raise of MetS (and consequently NAFLD) in the next decade, we can expect more donors with fatty liver disease [3, 8, 9], and consequently, a great proportion of potential organ donors will be rejected for LT use [3, 8]. Third, NAFLD patients often have multiple comorbidities, thus making LT a challenging procedure for them. Finally, in the post-LT setting, there are several challenging issues for NAFLD such as de novo NAFLD or recurrent NAFLD, as well as the risk for CKD and CVD [3].

With the help of LT, survival of patients with liver failure (acute or secondarily to cirrhosis) as well as those with HCC has significantly improved. Due to the progress in transplant surgery and in modern immunosuppressive therapy, early post-LT morbidity and mortality has decreased. Consequently, the focus of transplant doctors is changing to long-term complications, such as effects of donor liver steatosis, MetS and its associated complications, NAFLD, CVD, and CKD, as well as malignancy on the graft and recipient outcome [3, 9, 10]. Due to high rate of MetS and its individual components in the post-LT setting (mainly due to immunosuppressive medications), liver transplant recipients (LTR) have a high risk of graft steatosis and fibrosis (i.e., de novo or recurrent NAFLD). According to the data, MetS affects one out of every two LTR and accounts for up to 42% of CVD-related mortality [9, 11, 12]. Therefore, early recognition of graft steatosis and fibrosis are key issues to prevent adverse outcomes. Although liver biopsy (LB) is still the gold standard for the detection of steatosis, inflammation, and fibrosis, it is an invasive procedure, and LTR can be reluctant to undergo repetitive protocol biopsies [6]. In general population, non-invasive methods for steatosis and fibrosis detection and staging, such as transient elastography (TE) with a controlled attenuation parameter (CAP), have gained popularity in the last 5–10 years [3, 6]. Recently, study data revealed CAP and liver stiffness measurement (LSM) are the good methods for assessment of steatosis and fibrosis in NAFLD patients [13].

According to our best knowledge, there are only two studies that investigated the use of TE with CAP in the post-LT setting [6, 7]. Therefore, the aim of our study was to investigate the prevalence and risk factors of increased CAP and LSM as surrogate markers of liver steatosis and fibrosis in the Croatian Transplant Center that has one of the highest LT rates in the world.

2. Patients and Methods

2.1. Patients. In this prospective, cross-sectional study, we have evaluated 175 LTRs with a functioning graft for more than one year who came for regular outpatient examinations to the Department of Gastroenterology, University Hospital (UH) Merkur, Zagreb, Croatia, during the 10-month period between October 2019 and August 2020. All included LTRs were at least 18 years old at the time of TE measurements, while recipients with pregnancy, elevation of aminotransferases >5 times the upper limit of normal, as well as those with cholestasis, those with an excessive alcohol consumption (>20 g per day for men and >10 g per day for women), those with failed TE measurements, and those with missing data were not a part of this analysis. Additionally, recipients with malignancy, ascites, right-side heart failure, and valvular heart disease were excluded as well. The study was performed in accordance with the ethical guidelines of the Helsinki and was approved by the ethics committee of UH Merkur.

2.2. Objectives. The primary objective of this study was to evaluate the prevalence of increased CAP and LSM as surrogate markers of liver steatosis and fibrosis in LTR. Secondary objectives were to determine the predictors of increased CAP and LSM in population of LTR.

2.3. Clinical and Laboratory Data

2.3.1. Recipients and Donor's Data. After the surgical procedure of LT, all recipients were managed in the intensive care unit (ICU) with a standard triple immunosuppressive regimen (corticosteroids, mycophenolate mofetil, and calcineurin inhibitors) as well as postoperative antibiotic therapy and valganciclovir according to the CMV status.

The following recipients' data were analyzed in this study: age, age at LT, gender, aetiologias of ESLD, type of immunosuppressive regimen, recipient's age at the time of TE measurements, time from LT to TE examination, and presence of MetS components (diabetes mellitus, hypertension, obesity, and dyslipidemia). Donor age and body mass index (BMI) were analyzed as well. Obesity was defined

as BMI ≥30 kg/m^2. Hypertension was defined in LTR with a blood pressure ≥130/80 mm Hg or using antihypertensive medications, while diabetes as fasting glucose ≥7.1 mmol/L or use of at least 1 oral hypoglycemic drug or insulin. Finally, dyslipidemia was defined by positive medical history, using of lipid-lowering drugs, or if the serum total cholesterol level was ≥5.2 mmol/L, serum triglyceride (TG) level ≥1.7 mmol/L, and serum high-density lipoprotein (HDL) cholesterol level ≥3.4 mmol/L. Relevant clinical details were obtained from all patients at the time of TE measurements. Laboratory data (using standard laboratory methods) included complete blood cell count, liver tests (total bilirubin, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP)), lipidogram (total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides), glucose, and high sensitivity C-reactive protein (hs-CRP).

2.4. Transient Elastography. All patients underwent TE measurements after overnight fasting using FibroScan® 502 Touch (Echosens, Paris, France), which was performed using M or XL probe by a certified investigator. Only cases with 10 successful measurements were included in this study. Examinations with an interquartile range/median ratio >30% were excluded because of unreliable results. CAP was used as a surrogate parameter for graft steatosis and was expressed in dB/m, while LSM was used as a surrogate parameter for graft fibrosis and was expressed in kPa. Measurements were performed in decubital position with right arm placed above the patients' head, in the neutral respiratory position, while suspending breathing. We did not have adverse events related to the use of the FibroScan device.

Accordingly, TE patients were considered to have hepatic steatosis if the controlled attenuation parameter (CAP) was ≥238 dB [14]. Severe steatosis was considered if the CAP was ≥290 dB/m. Moderate liver fibrosis (≥*F*2) was considered as a LSM ≥7 kPa and advanced fibrosis (≥*F*3) if LSM was ≥9.6 kPa using the M probe or ≥9.3 kPa using the XL probe [15, 16].

2.5. Statistical Analysis. Categorical variables are shown as percentages and continuous variables as means with standard deviation or medians with interquartile range (25th and 75th percentiles) depending on the distribution. Distribution was assessed using the D'Agostino-Pearson test and graphically. Distribution relationship between categorical variables values was tested using the χ^2-test and if necessary, Fisher's exact test. Difference between two continuous variables was tested using the two-way *t*-test for parametric or Mann–Whitney *U* test for nonparametric analysis. Multivariable logistical regression analyses were conducted to identify patient characteristics independently associated with liver steatosis and fibrosis according to the transient elastography. Univariate analysis was first performed on each variable of the independent variables to select variables for the multivariable analyses. Those factors with a p value < 0.5 in the univariate analyses were selected as candidate variables for backward multivariable logistical regressions. All the statistical analyses were performed using SPSS V.22.0 (SPSS Inc., Chicago, Illinois, USA). Statistical tests were two-tailed, and significance was set at 0.05.

3. Results

3.1. Patient Characteristics. A total of 175 patients were included in this study and underwent FibroScan assessment. The mean age of the total study population was 61 (53–65), and 68% (119/175) of them were male. The average BMI of the study population was 28.4 (23.69–31.99) kg/m^2, while the prevalence of obesity was 34.28% (60/175). In FibroScan assessment, M probe was used in 121 (69.14%) and XL probe in 54 (30.85%) patients, respectively. Furthermore, 64.00% (112/175) patients had hypertension, 38.28% (67/175) diabetes, and 58.85% (103/175) hyperlipidemia. Overall, 118 patients (67.42%) had the echobright liver on abdominal ultrasound (i.e., liver steatosis based on abdominal ultrasound finding).

3.2. Prevalence and Predictors of Liver Steatosis and Severe Liver Steatosis. In our population, the prevalence of liver steatosis was 68.57% (120/55), while the prevalence of severe liver steatosis was 46.85% (82/93). Patient characteristics with and without increased CAP are shown in Table 1, while patient characteristics with and without severe liver steatosis are shown in Table 2.

Patients with increased liver steatosis were more often males and had most often alcoholic liver disease as the pretransplant cause of liver cirrhosis, higher proportion of arterial hypertension, higher levels of blood glucose, GGT, triglycerides, total cholesterol, and LDL, and lower levels of HDL. Also, patients with increased liver steatosis had higher levels of LSM (7.2 vs. 5.8 kPa, $p = 0.012$) and higher time from LT to TE (Table 1). On multivariate analysis, independent factors associated with liver steatosis were male gender and total cholesterol as positive predictor and HDL as negative predictor (Table 3).

Furthermore, patients with severe steatosis were older and had higher BMI (30.44 vs. 26.51 kg/m^2, $p = 0.038$) and consequently higher proportion of obesity, diabetes, blood glucose, and LSM levels (7.4 vs. 6.7 kPa, $p = 0.019$) (Table 2). On multivariate analysis, independent factors positively associated with severe liver steatosis were higher BMI and higher triglyceride levels (Table 4).

3.3. Prevalence and Predictors of Moderate Liver Fibrosis and Advanced Liver Fibrosis. In our population, the prevalence of moderate liver fibrosis was 54.85 (96/81), while the prevalence of advanced liver fibrosis was 24% (42/135). Patients characteristics with and without moderate fibrosis are shown in Table 5, while patient characteristics with and without advanced fibrosis are shown in Table 6.

Patients with moderate liver fibrosis had a higher prevalence of arterial hypertension and higher levels of ALT, AST, GGT, and CAP (298 vs. 267 dB, $p = 0.004$) in addition to longer time from performance of LT to TE. Also, patients with moderate fibrosis were more often treated with

Table 1: Comparison of groups with and without steatosis (elevated CAP ≥238 dB).

Variables	Steatosis CAP ≥238 dB ($n = 120$)	No steatosis CAP <238 dB ($n = 55$)	p value
Age at LT, years (IQR)	55 (50–61)	57 (43–60)	0.286
Age at TE, years (IQR)	61 (54–65)	60 (46–65)	0.131
Donor age, years	60 (48–70)	57 (41–68)	0.596
Male, % (n)	75.83 (91)	52.72 (29)	0.006*
Cause of liver disease, % (n)			
Autoimmune liver disease	6.67 (8)	0.2 (11)	
NAFLD	0.83 (1)	0.0 (0)	
Alcoholic liver disease	37.5 (45)	18.18 (10)	0.005*
HCV	5.0 (6)	0.0 (0)	
Others	50.0 (60)	61.8 (34)	
BMI, kg/m^2 (IQR)	28.76 (23–32)	26.67 (24–30)	0.402
BMI category, % (n)			
Normal <25	37.5 (45)	36.4 (20)	
Overweight 25–29.9	23.3 (28)	40.0 (22)	0.246
Obese ≥30	39.2 (47)	23.6 (13)	
Donors BMI, kg/m^2 (IQR)	26.23 (24–28)	26.03 (24–28)	0.911
Donors BMI category, % (n)			
Normal <25	40.0 (48)	34.54 (19)	
Overweight 25–29.9	44.2 (53)	52.73 (29)	0.627
Obese ≥30	15.8 (19)	12.73 (7)	
Hypertension, % (n)	69.75 (83)	52.73 (29)	0.041*
Diabetes, % (n)	43.70 (52)	27.27 (15)	0.063
Thrombocytes x10^9/L	167 (135–226)	170 (136–217)	0.632
Glucose, mmol/L (IQR)	6.4 (6–8)	5.8 (5–6)	<0.001*
Total bilirubin, mg/dL (IQR)	16 (12–21)	15 (12–26)	0.558
ALT, U/L (IQR)	29 (20–39)	26 (17–34)	0.186
AST, U/L (IQR)	28 (23–40)	28 (22–37)	0.598
GGT, U/L (IQR)	41 (24–94)	31 (17–84)	0.041*
Triglyceride, mmol/L (IQR)	1.37 (1.1–2.0)	1.03 (0.8–1.4)	<0.001*
Total cholesterol, mmol/L (IQR)	5.1 (4.5–5.9)	4.7 (4.1–5.5)	0.025*
LDL, mmol/L (IQR)	3.1 (2.6–3.7)	2.8 (2.1–3.5)	0.016*
HDL, mmol/L (IQR)	1.3 (1.1–1.6)	1.4 (1.2–1.7)	0.033*
CRP, mg/L (IQR)	3.4 (2–6)	2.3 (1–4)	0.066
LSM, kPa (IQR)	7.2 (6.0–9.0)	5.8 (4.5–9.4)	0.012*
Immunosuppression, % (n)			
Tacrolimus	67.5 (81)	76.4 (42)	
Cyclosporine	32.5 (39)	21.8 (12)	
Prednisone	3.3 (4)	7.3 (4)	0.311
mTOR inhibitor	(0)	1.8 (1)	
Mycophenolate mofetil	70.0 (84)	65.5 (36)	
Azathioprine	(0)	5.5 (3)	
Time from LT to TE, years (IQR)	4 (3–6)	3 (2–5)	0.033*

*LT, liver transplantation; NAFLD, nonalcoholic fatty liver disease; HCV, hepatitis C virus infection; BMI, body mass index; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; LSM, liver stiffness measurement; TE, transient elastography; CAP, controlled attenuation parameter.

cyclosporine and mycophenolate mofetil and less often with tacrolimus (Table 5). On multivariate analysis, independent factors positively associated with moderate fibrosis were GGT and CAP (Table 7).

Furthermore, patients with advanced fibrosis had higher levels of total bilirubin, AST, ALT, GGT, and LDM and longer time from LT to TE. As in moderate fibrosis, patients with advanced fibrosis were also more often treated with cyclosporine and mycophenolate mofetil compared to patients without advanced fibrosis (Table 6). On multivariate analysis, an independent factor positively associated with advanced fibrosis was GGT (Table 8).

4. Discussion

To the best of our knowledge, this is the third observational study [6, 7] aimed to investigate graft injury noninvasively with CAP and LSM obtained by FibroScan as a surrogate marker of steatosis and fibrosis, which reveals a high prevalence of post-LT steatosis that was associated with the MetS and liver graft fibrosis. NAFLD affects about 25% of the total population, and it is closely related to diabetes mellitus, hypertension, dyslipidemia, and obesity, i.e., the MetS components. Today, we know that NAFLD is the liver manifestation of MetS. Metabolic syndrome and its

Table 2: Comparison of groups with and without severe steatosis (elevated CAP ≥290 dB).

Variables	Severe steatosis CAP ≥290 dB ($n = 82$)	No severe steatosis CAP <290 dB ($n = 93$)	p value
Age at LT, years (IQR)	55 (50–61)	55 (45–61)	0.412
Age at TE, years (IQR)	64 (55–69)	60 (51–65)	0.039*
Donor age, years	57 (42–68)	59 (45–67)	0.711
Male, % (n)	71.95 (59)	64.52 (60)	0.374
Cause of liver disease, % (n)			
Autoimmune liver disease	8.54 (7)	12.90 (12)	
NAFLD	1.22 (1)	(0)	
Alcoholic liver disease	39.02 (32)	24.73 (23)	0.211
HCV	3.66 (3)	3.23 (3)	
Others	47.56 (39)	59.14 (55)	
BMI, kg/m^2 (IQR)	30.44 (26–34)	26.51 (23–30)	0.038*
BMI category, % (n)			
Normal <25	26.8 (22)	43.0 (40)	
Overweight 25–29.9	26.8 (22)	31.2 (29)	0.045*
Obese ≥30	46.4 (38)	25.8 (24)	
Donors BMI, kg/m^2 (IQR)	26.57 (24–29)	26.23 (24–28)	0.877
Donors BMI category, % (n)			
Normal <25	40.2 (33)	35.5 (33)	
Overweight 25–29.9	41.5 (34)	52.7 (49)	0.266
Obese ≥30	18.3 (15)	11.8 (11)	
Hypertension, % (n)	71.95 (59)	58.06 (54)	0.079
Diabetes, % (n)	50.00 (41)	27.96 (26)	0.005*
Thrombocytes x10^9/L	166 (134–218)	170 (136–228)	0.652
Glucose, mmol/L (IQR)	6.4 (6–8)	6 (5–7)	0.018*
Total bilirubin, mmol/L (IQR)	16.5 (12–22)	14.0 (12.22)	0.732
ALT, U/L (IQR)	29.5 (20–39)	26.0 (18–36)	0.056
AST, U/L (IQR)	27.5 (22–43)	28 (23–39)	0.319
GGT, U/L (IQR)	42 (24–99)	36 (21–80)	0.191
Triglyceride, mmol/L (IQR)	1.4 (1.1–2.1)	1.1 (0.9–1.5)	0.004
Total cholesterol, mmol/L (IQR)	5.2 (4.5–6)	4.9 (4.4–5.6)	0.168
LDL, mmol/L (IQR)	3.1 (2.5–3.7)	3 (2.4–3.5)	0.340
HDL, mmol/L (IQR)	1.26 (1.0–1.6)	1.34 (1.1–1.6)	0.227
CRP, mg/L (IQR)	3.7 (2–6)	2.5 (1–5)	0.755
LSM, kPa (IQR)	7.4 (6.3–9.2)	6.7 (5.0–9.0)	0.019*
Immunosuppression, % (n)			
Tacrolimus	68.3 (56)	72.0 (67)	
Cyclosporine	31.7 (26)	28.0 (26)	
Prednisone	1.2 (1)	8.0 (7)	0.356
mTOR inhibitor	(0)	1.1 (1)	
Mycophenolate mofetil	65.9 (54)	72.0 (67)	
Azathioprine	(0)	3.2 (3)	
Time from LT to TE, years (IQR)	5 (3–6)	3 (2–5)	0.099

*LT, liver transplantation; NAFLD, nonalcoholic fatty liver disease; HCV, hepatitis C virus infection; BMI, body mass index; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; LSM, liver stiffness measurement; TE, transient elastography.

individual components often develop in the post-LT setting, and immunosuppressive therapy is the main trigger that promotes individual MetS components development [5–7, 9]. According to our results, the prevalence of diabetes, hypertension, obesity, and dyslipidemia was 38.3%, 64%, 34.3%, and 58.85%, respectively. Steatosis after LT has attracted increasing research interest during the last decade. Few authors have published their retrospective studies in which steatosis was defined by LB [17–19]. In our cohort of LTRs, the prevalence of increased CAP values (i.e., steatosis) was 68.57%, while the prevalence of severe liver steatosis was 46.85%. As it was mentioned, there are only two more studies to data that investigated the usefulness of TE with CAP for steatosis and fibrosis detection in the post-LT setting. In the study by Karlas et al. [6], the prevalence of steatosis was 44%, while the prevalence of advanced steatosis was 24%, which is lower than in our study. This can be explained by the fact that in the study by Karlas et al. [6], CAP was not available using the XL probe, and thus, only the M probe was used [6]. Our results closely resembled the results from Chayanupatkul et al. [7], in which TE with CAP was also used as a method for post-LT NAFLD. They reported that 70% of their LTRs had liver steatosis noted on TE; 7.3% LTRs had mild steatosis, 34.7%

Table 3: Univariate and multivariate analysis of predictors of steatosis (elevated CAP ≥238 dB).

Variables	Univariate		Multivariate	
	OR (95% CI)	p value	OR (95% CI)	p value
Age at LT, years	1.04 (1.01–1.07)	0.026*	0.94 (0.91–1.09)	0.445
Age at TE, years	1.04 (1.01–1.07)	0.008*	1.05 (0.90–1.22)	0.494
Donor age, years	0.99 (0.97–1.01)	0.462		
Gender (female ref.)	2.78 (1.42–5.46)	0.003*	2.73 (1.25–5.94)	0.011*
Cause of liver disease				
Autoimmune liver disease	0.29 (0.11–0.76)	0.012*	0.50 (0.16–1.57)	0.239
Alcoholic liver disease	2.74 (1.26–5.96)	0.011*	1.61 (0.67–4.03)	0.277
Others	0.61 (0.32–1.17)	0.134		
BMI, kg/m^2	1.03 (0.94–1.13)	0.509		
Donors BMI, kg/m^2	1.01 (0.93–1.10)	0.867		
Hypertension	2.08 (1.07–3.99)	0.031*	1.23 (0.52–2.90)	0.631
Diabetes	2.67 (1.03–4.15)	0.040*	0.85 (0.32–2.23)	0.749
Thrombocytes x10^9/L	1.01 (0.99–1.02)	0.545		
Glucose, mmol/L	1.37 (1.06–1.78)	0.017*	1.07 (0.81–1.42)	0.605
Total bilirubin, mmol/L	1.98 (0.95–1.01)	0.206		
ALT, U/L	1.01 (0.99–1.02)	0.592		
AST, U/L	1.01 (0.99–1.02)	0.385		
GGT, U/L	1.01 (0.99–1.02)	0.332		
Triglyceride, mmol/L	2.67 (1.46–4.90)	0.002*	1.19 (0.16–8.54)	0.862
Total cholesterol, mmol/L	1.47 (1.07–2.02)	0.018*	7.46 (1.78–31.15)	0.006*
LDL, mmol/L	1.61 (1.10–2.35)	0.015*	1.21 (0.91–1.46)	0.065
HDL, mmol/L	0.39 (0.17–0.91)	0.023*	0.09 (0.02–0.42)	0.002*
CRP, mg/L	0.99 (0.96–1.01)	0.305		
LSM, kPa	1.03 (0.97–1.09)	0.287		
Time from LT to TE, years	1.14 (0.99–1.30)	0.065		

*LT, liver transplantation; NAFLD, nonalcoholic fatty liver disease; HCV, hepatitis C virus infection; BMI, body mass index; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; LSM, liver stiffness measurement; TE, transient elastography.

Table 4: Univariate and multivariate analysis of predictors of severe steatosis (elevated CAP ≥290 dB).

Variables	Univariate		Multivariate	
	OR (95% CI)	p value	OR (95% CI)	p value
Age at LT, years	1.02 (0.99–1.05)	0.115		
Age at TE, years	1.03 (1.01–1.06)	0.049*	1.01 (0.96–1.07)	0.494
Donor age, years	0.99 (0.97–1.02)	0.574		
Gender (female ref.)	1.48 (0.77–2.82)	0.240		
Cause of liver disease				
Autoimmune liver disease	0.64 (0.24–1.71)	0.372	1.52 (0.91–1.83)	0.984
Alcoholic liver disease	1.99 (1.04–3.80)	0.038*		
HCV	1.15 (0.23–5.88)	0.863		
Others	0.61 (0.33–1.11)	0.108		
BMI, kg/m^2	1.10 (1.01–1.20)	0.042*	2.77 (1.34–3.85)	0.048*
Donors BMI, kg/m^2	1.01 (0.93–1.09)	0.836		
Hypertension	0.10 (0.01–1.54)	0.100		
Diabetes	2.64 (1.41–4.95)	0.002*	1.48 (1.21–1.85)	0.044*
Thrombocytes x10^9/L	0.99 (0.98–1.01)	0.621		
Glucose, mmol/L	1.21 (1.02–1.45)	0.033*	1.11 (0.95–1.30)	0.368
Total bilirubin, mmol/L	1.01 (0.98–1.04)	0.718		
ALT, U/L	1.01 (0.99–1.02)	0.534		
AST, U/L	1.01 (0.99–1.02)	0.309		
GGT, U/L	1.01 (0.98–1.02)	0.184		
Triglyceride, mmol/L	1.79 (1.18–2.69)	0.006*	1.63 (1.15–2.59)	0.041*
Total cholesterol, mmol/L	1.23 (0.93–1.63)	0.146		
LDL, mmol/L	1.19 (0.86–1.66)	0.290		
HDL, mmol/L	0.60 (0.27–1.34)	0.209		
CRP, mg/L	0.99 (0.97–1.02)	0.770		
LSM, kPa	1.02 (0.97–1.07)	0.410		
Time from LT to TE, years	1.09 (0.98–1.23)	0.104		

*LT, liver transplantation; NAFLD, nonalcoholic fatty liver disease; HCV, hepatitis C virus infection; BMI, body mass index; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; LSM, liver stiffness measurement; TE, transient elastography.

Table 5: Comparison of groups with and without moderate fibrosis.

Variables	Moderate fibrosis, $N = 96$	No moderate fibrosis, $N = 81$	p value
Age at LT, years (IQR)	55 (46–60)	56 (49–62)	0.288
Age at TE, years (IQR)	61 (53–64)	60 (53–66)	0.818
Donor age, years	57 (44–68)	60 (44–67)	0.971
Male, % (*n*)	68.1 (64)	68.7 (55)	0.945
Cause of liver disease, % (*n*)			
Autoimmune liver disease	8.3 (8)	14.8 (12)	
NAFLD	0	1.2 (1)	
Alcoholic liver disease	36.5 (35)	27.2 (22)	0.280
HCV	2.1 (2)	4.9 (4)	
Others	53.1 (51)	51.9 (42)	
BMI, kg/m^2 (IQR)	28.4 (24–32)	27.5 (23–32)	0.870
BMI category, % (*n*)			
Normal <25	35.4 (34)	38.3 (31)	0.737
Overweight 25–29.9	32.3 (31)	23.4 (19)	
Obese ≥30	32.3 (31)	38.3 (31)	
Donors BMI, kg/m^2 (IQR)	26.3 (25–29)	26 (23–28)	0.132
Donors BMI category, % (n)			
Normal <25	31.2 (30)	44.4 (36)	
Overweight 25–29.9	55.2 (53)	39.5 (32)	0.105
Obese ≥30	13.5 (13)	16.0 (13)	
Hypertension, % (*n*)	72.9 (70)	55.6 (45)	0.024*
Diabetes, % (*n*)	41.7 (40)	35.8 (29)	0.521
Thrombocytes x10^9/L	161 (129–221)	170 (139–219)	0.540
Glucose, mmol/L (IQR)	6.3 (5–8)	6 (5.4–6.9)	0.152
Total bilirubin, mg/dL (IQR)	17 (12–24)	15 (11–19)	0.173
ALT, U/L (IQR)	31 (23–49)	24 (17–33)	<0.001*
AST, U/L (IQR)	31 (23–48)	26 (21–34)	0.001*
GGT, U/L (IQR)	53 (28–127)	29 (17–43)	<0.001*
Triglyceride, mmol/L (IQR)	1.32 (1–1.9)	1.16 (0.9–1.7)	0.095
Total cholesterol, mmol/L (IQR)	5.1 (4.4–5.7)	5.2 (4.5–6.1)	0.572
LDL, mmol/L (IQR)	3 (2.4–3.5)	3.2 (2.5–3.8)	0.133
HDL, mmol/L (IQR)	1.3 (1.1–1.6)	1.3 (1.1–1.6)	0.692
CRP, mg/L (IQR)	3.3 (2–6)	3.1 (1.9–5.8)	0.892
CAP, dB (IQR)	298 (250–334)	267 (203–310)	0.004*
Immunosuppression, % (*n*)			
Tacrolimus	62.5 (60)	80.2 (65)	
Cyclosporine	37.2 (35)	21.5 (17)	
Prednisone	5.2 (5)	3.7 (3)	0.016*
mTOR inhibitor	1.0 (1)	(0)	
Mycophenolate mofetil	74.0 (71)	61.7 (50)	
Azathioprine	1.0 (1)	2.5 (2)	
Time from LT to TE, years (IQR)	5 (3–6)	3 (2–5)	0.008*

*LT, liver transplantation; NAFLD, nonalcoholic fatty liver disease; HCV, hepatitis C virus infection; BMI, body mass index; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; CAP, controlled attenuation parameter; TE, transient elastography.

had moderate steatosis, and 28.0% of LTRs had severe steatosis. According to the literature, the prevalence of steatosis varies across studies, which is probably the consequence of different criteria and methods that were used for steatosis definition. For example, Dumortier et al. [18] analyzed patients who were mainly transplanted for alcoholic liver disease and found that a histological diagnosis of steatosis was present in 131 (31.1%) of the remaining 421 LTRs. Similarly, another biopsy-based study reported the prevalence of allograft steatosis of 40% [19]. Of this, 58% LTRs had mild steatosis while 42% had moderate steatosis [19]. A study from Mayo Clinic reported the prevalence of steatosis of 48% after 10 years post-LT [20]. In our study, the prevalence of steatosis was higher than the aforementioned studies but quite similar to results by Chayanupatkul et al. [7], which also used TE as a method for steatosis assessment in the post-LT setting. However, according to the largest study with protocol biopsy in the context of post-LT NAFLD, NAFLD was present in 67% of the patients with de novo NAFLD and in 100% of the patients with recurrent NAFLD one year after LT [20].

In line with the study by Chayanupatkul et al. [7], in our study, male gender, older age, and alcoholic liver disease as an indication for LT were the risk factors for post-LT

Table 6: Comparison of groups with and without advanced fibrosis.

Variables	Advanced fibrosis, $N = 42$	No advanced fibrosis, $N = 135$	p value
Age at LT, years (IQR)	53 (44–60)	56 (50–61)	0.061
Age at TE, years (IQR)	58 (51–64)	61 (54–65)	0.159
Donor age, years	59 (46–70)	57 (43–67)	0.806
Male, % (*n*)	71.4 (30)	67.4 (89)	0.768
Cause of liver disease, % (*n*)			
Autoimmune liver disease	7.1 (3)	12.6 (17)	
NAFLD	0	0.7 (1)	
Alcoholic liver disease	40.5 (17)	29.6 (40)	0.578
HCV	4.8 (2)	3.0 (4)	
Others	47.6 (20)	54.1 (73)	
BMI, kg/m^2 (IQR)	29.35 (23–32)	27 (24–32)	0.738
BMI category, % (*n*)			
Normal <25	40.5 (17)	34.8 (47)	
Overweight 25–29.9	19.0 (8)	32.6 (44)	0.487
Obese ≥30	40.5 (17)	32.6 (44)	
Donors BMI, kg/m^2 (IQR)	26.54 (25–29)	26 (24–28)	0.312
Donors BMI category, % (*n*)			
Normal <25	31.0 (13)	39.3 (53)	
Overweight 25–29.9	52.4 (22)	46.7 (63)	0.620
Obese ≥30	16.7 (7)	14.1 (19)	
Hypertension, % (*n*)	76.2 (32)	61.5 (83)	0.119
Diabetes, % (*n*)	45.2 (19)	37.0 (50)	0.441
Thrombocytes x10^9/L	145 (106–221)	170 (144–221)	0.082
Glucose, mmol/L (IQR)	6.2 (6–8)	6.1 (5–7)	0.240
Total bilirubin, mg/dL (IQR)	18 (14–26)	15 (12–20)	0.005*
ALT, U/L (IQR)	33 (24–60)	26 (18–35)	<0.001*
AST, U/L (IQR)	43 (25–72)	26 (21–35)	<0.001*
GGT, U/L (IQR)	83 (40–173)	31 (21–65)	<0.001*
Triglyceride, mmol/L (IQR)	1.43 (1.1–1.9)	1.2 (0.9–1.8)	0.066
Total cholesterol, mmol/L (IQR)	5.1 (4.3–5.5)	5.1 (4.5–6)	0.282
LDL, mmol/L (IQR)	3.1 (2.6–3.7)	2.9 (2.3–3.4)	0.048*
HDL, mmol/L (IQR)	1.2 (1–1.7)	1.3 (1.1–1.6)	0.429
CRP, mg/L (IQR)	4.4 (2–7)	3.1 (1.8–5.6)	0.368
CAP, dB (IQR)	285 (213–341)	277 (222–315)	0.469
Immunosuppression, % (*n*)			
Tacrolimus	50.0 (21)	77.0 (104)	
Cyclosporine	52.5 (21)	23.3 (31)	
Prednisone	2.4 (1)	5.2 (7)	<0.001*
mTOR inhibitor	2.4 (1)	0	
Mycophenolate mofetil	78.6 (33)	65.2 (88)	
Azathioprine	2.4 (1)	1.5 (2)	
Time from LT to TE, years (IQR)	5 (3–6)	4 (2–6)	0.015*

*LT, liver transplantation; NAFLD, nonalcoholic fatty liver disease; HCV, hepatitis C virus infection; BMI, body mass index; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; CAP, controlled attenuation parameter; TE, transient elastography.

steatosis. Contrary to our results, a recent study reported that younger age at time of LT was a risk factor for post-LT steatosis [21], which may be explained by the type of LT [5–7]. Namely, in our study, we have had deceased donors, while Miyaaki [22] et al. had living donors. Recently, we have shown that CAP values were strongly associated with all components of MetS [23–25] in the pre-LT setting. In line with our previous results [23–25], this study confirms that CAP as a surrogate marker of steatosis is related to MetS components also in the post-LT setting. Namely, in our study, CAP was associated with hypertension, higher levels of glucose in blood, and dyslipidemia. Moreover, LTRs with severe steatosis (CAP ≥ 290 db/m) were more obese and had a higher prevalence of diabetes, while independent predictors of severe steatosis were obesity and dyslipidemia. Other biopsy-proven studies confirmed that steatosis post-LT is related to MetS and its individual components, and a study that used CAP, as well as our study, for steatosis detections, also reported that CAP is related to MetS components [6, 18]. Interestingly, in our study, liver enzymes were not related to higher CAP values (severe steatosis), which is similar to results of other authors [7] and to earlier observation that about 50% of NAFLD patients in the pre-LT setting have normal liver tests [23–25].

Table 7: Univariate and multivariate analysis of predictors of moderate fibrosis.

Variables	Univariate		Multivariate	
	OR (95% CI)	p value	OR (95% CI)	p value
Age at LT, years	0.99 (0.96–1.02)	0.521		
Age at TE, years	1.00 (0.97–1.03)	0.972		
Donor age, years	1.01 (0.98–1.02)	0.535		
Gender (female ref.)	0.96 (0.51–1.84)	0.925		
Cause of liver disease, % (n)				
Autoimmune liver disease	0.52 (0.20–1.35)	0.180		
Alcoholic liver disease	1.54 (0.81–2.92)	0.188		
HCV	0.41 (0.07–2.30)	0.310		
Others	1.05 (0.58–1.90)	0.866		
BMI, kg/m^2	1.02 (0.93–1.11)	0.692		
Donors BMI, kg/m^2	1.05 (0.97–1.14)	0.212		
Hypertension, % (n)	2.15 (1.15–4.04)	0.017		
Diabetes, % (n)	1.28 (0.70–2.36)	0.426		
Thrombocytes x10^9/L	0.99 (0.98–1.01)	0.844		
Glucose, mmol/L	1.11 (0.96–1.28)	0.171		
Total bilirubin, mmol/L	1.01 (0.98–1.04)	0.433		
ALT, U/L	1.02 (1.01–1.03)	0.019*	1.01 (0.97–1.03)	0.717
AST, U/L	1.02 (1.01–1.04)	0.013*	1.01 (0.98–1.02)	0.937
GGT, U/L	1.02 (1.01–1.03)	0.001*	1.03 (1.01–1.05)	0.001*
Triglyceride, mmol/L	1.34 (0.92–1.94)	0.126		
Total cholesterol, mmol/L	0.91 (0.69–1.20)	0.495		
LDL, mmol/L	0.77 (0.55–1.07)	0.125		
HDL, mmol/L	1.15 (0.52–2.54)	0.726		
CRP, mg/L	0.98 (0.94–1.02)	0.325		
CAP, db/m	1.02 (1.01–1.03)	0.003*	1.08 (1.02–1.15)	0.010*
Time from LT to TE, years	1.15 (1.02–1.30)	0.024*	1.06 (0.92–1.22)	0.403

*LT, liver transplantation; NAFLD, nonalcoholic fatty liver disease; HCV, hepatitis C virus infection; BMI, body mass index; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; CAP, controlled attenuation parameter; TE, transient elastography.

In the second part of our analysis, we have investigated the prevalence of increased LSM as a surrogate marker of liver fibrosis. In our population, the prevalence of moderate liver fibrosis was 54.85%, while the prevalence of advanced liver fibrosis was 24%. These results may be a consequence of a higher rate of MetS and its individual components in our cohort of LTRs. In contrast to the study by Chayanupatkul et al. [7], in our study, AST, ALT, and GGT were associated with moderate and advanced fibrosis, while in multivariate analysis, GGT was an independent predictor of moderate and advanced fibrosis. However, we have to keep in mind that various factors can influence the increased level of liver enzymes in LTRs. Second, we still do not know what is the true "normal" range for ALT in this population of patients [7]; thus, we cannot reliably use ALT as a marker for further studies when it comes to steatosis (i.e., NAFLD) screening in the context of LTRs [7]. Hypertension was related to the moderate fibrosis, which is in line with the data from the pre-LT setting where hypertension is a risk factor for fibrosis progression [23–25]. Interestingly, although this association did not persist in multivariate analysis, LTRs with moderate and advanced fibrosis were more often treated with cyclosporine and less often with tacrolimus. In contrast to our result, in a study by Dumortier et al. [18], one-third of their analyzed LTRs had perisinusoidal fibrosis, and 4% of LTRs had NASH. Factors that were related to the post-LT steatosis were MetS and its individual components, tacrolimus-based immunosuppressive therapy, alcoholic liver disease as the primary indication for LT, and liver graft steatosis [18]. Our result may partially explain the fact that, in our transplant center, we keep the tacrolimus concentrations in blood within the lowest possible range; thus, their negative effect on the kidneys, hypertension, and diabetes is minimalized. Furthermore, prospective studies that will investigate the influence of immunosuppressive therapy on CAP and LSM values are needed.

The exact role of post-LT steatosis and effects of pre-transplant donor steatosis on it are not completely elucidated yet. According to our results and similar to other two studies [6, 7] that used TE with CAP in the post-LT setting, CAP was associated with increased LSM (i.e., fibrosis). Although, in recent biopsy-based study in the pre-LT setting [13], LSM measurements have not been affected by CAP (steatosis); our results as well as results of other two studies [6, 7] indicate an association of allograft steatosis and fibrosis. Namely, we cannot rule out an impact of inflammation (i.e., steatohepatitis) on our measurements because ongoing graft inflammation could be associated with increased LSM even in cases where significant and advanced fibrosis is not present. Regarding our previous experience with TE with CAP, we strongly believe that elevated LSM in LTRs could be a parameter for previous or ongoing graft damage [6, 23–25]. Similar results were observed by Karlas et al. five years ago [6]. As it was mentioned, the rule of post-

Table 8: Univariate and multivariate analysis of predictors of advanced fibrosis.

Variables	Univariate		Multivariate	
	OR (95% CI)	p value	OR (95% CI)	p value
Age at LT, years	0.98 (0.95–1.01)	0.146		
Age at TE, years	0.98 (0.95–1.02)	0.309		
Donor age, years	1.01 (0.98–1.02)	0.580		
Gender (female ref.)	1.21 (0.56–2.59)	0.627		
Cause of liver disease, % (n)				
Autoimmune liver disease	0.53 (0.15–1.92)	0.337		
Alcoholic liver disease	1.62 (0.79–3.31)	0.191		
HCV	1.64 (0.29–9.27)	0.577		
Others	0.77 (0.39–1.55)	0.465		
BMI, kg/m^2 (IQR)	1.03 (0.93–1.14)	0.541		
Donors BMI, kg/m^2 (IQR)	1.05 (0.95–1.15)	0.342		
Hypertension, % (n)	2.00 (0.91–4.42)	0.085		
Diabetes, % (n)	1.40 (0.70–2.83)	0.342		
Thrombocytes	0.99 (0.98–1.01)	0.468		
Glucose, mmol/L (IQR)	1.13 (0.99–1.28)	0.067		
Total bilirubin, mg/dL (IQR)	1.03 (1.01–1.07)	0.046*	1.01 (0.95–1.06)	0.962
ALT, U/L (IQR)	1.02 (1.01–1.03)	0.002*	0.98 (0.96–1.01)	0.360
AST, U/L (IQR)	1.04 (1.02–1.05)	<0.001*	1.03 (0.99–1.07)	0.064
GGT, U/L (IQR)	1.02 (1.01–1.04)	<0.001*	1.04 (1.01–1.06)	0.031*
Triglyceride, mmol/L (IQR)	1.46 (1.02–2.11)	0.047*		
Total cholesterol, mmol/L (IQR)	0.78 (0.56–1.06)	0.150		
LDL, mmol/L (IQR)	1.35 (1.02–.1.65)	0.040*	1.30 (0.90–1.64)	0.126
HDL, mmol/L (IQR)	0.91 (0.36–2.30)	0.837		
CRP, mg/L (IQR)	0.99 (0.95–1.03)	0.640		
CAP, db/m	1.00 (0.99–1.02)	0.491		
Time from LT to TE, years (IQR)	1.13 (0.99–1.27)	0.053		

*LT, liver transplantation; NAFLD, nonalcoholic fatty liver disease; HCV, hepatitis C virus infection; BMI, body mass index; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; CAP, controlled attenuation parameter; TE, transient elastography.

LT steatosis (i.e., NAFLD) is not completely investigated and understood. A recent study by Gitto et al. [26] reported that de novo NAFLD was associated with adverse CVD events and extrahepatic malignancy, and biopsy-proven NASH was related to the higher long-term LTRs mortality. Thus, but mainly regarding the results in the pre-LT setting, CAP as a surrogate marker of steatosis could become a growing clinical relevance for the follow-up of LTRs because it is easy to use, and it is a noninvasive method for steatosis detection [5–7, 9]. By using TE with CAP to detect and assess the degree of steatosis in LTRs, we could motivate transplant physicians to aggressively treat MetS and its individual components, obesity, diabetes, hypertension, and dyslipidemia [5–7, 9]. Therefore, further investigations in the post-LT setting should answer on the question whether monitoring the changes in the CAP and LSM could be useful for evaluating the treatment of the MetS and the effect of treatment of MetS and its components on de novo and recurrent NAFLD [5–7, 9]. Post-LT steatosis (i.e., NAFLD) is not only important for liver-related mortality but also for some extrahepatic diseases [9]. Namely, today, we know from the data from the pre-LT setting that NAFLD is a multisystem disease that is a risk factor for CVD, CKD, and diabetes type 2, as well as a risk factor for some malignancies such as colorectal cancer [1]. On the other hand, the high incidence of long-term complications after LT such as CKD and CVD suggests the need for a stratification model to identify LTRs at a high risk of developing CKD and CVD post-LT [5–7, 9]. Consequently, further investigations should answer on the question will early NAFLD recognition in the post-LT setting help us to identify those LTRs that are at high risk of CKD and CVD development. In this context, CAP as a surrogate marker of steatosis could have a role [9] because CAP, as a surrogate marker of NAFLD in the pre-LT setting, showed a correlation with cardiovascular risk and CKD [9, 27–30]. Considering this association, the question is whether patients with increased CAP and specifically an increased LSM could benefit from much earlier and much stronger screening for CVD and CKD [9]. We are questioning whether CAP and LSM could be a surrogate marker of subclinical atherosclerosis and consequent markers of increased CVD risk in the post-LT setting [9]. Further studies on this topic are needed. Earlier studies addressed the limitations of the M probe in patients with higher BMI, which led to the development of the XL probe that is specially designed for obese people [5, 27].

Additionally, earlier data addressed that graft fibrosis may in occur in high proportion of LTRs who have normal transaminase levels [5–7]. On the other hand, in our center as well as in many other transplant centers, protocol biopsies are not a part of standard care of LTRs. Consequently, LSM could be a good noninvasive method for the selection of those LTRs that are at risk and who need LB [5–7]. However, the optimal LSM cutoff for detecting each stage of liver fibrosis in the post-

LT setting has not been defined yet and need further studies [5]. Finally, by TE with CAP as a noninvasive method, we could routinely monitor steatosis and fibrosis progression in LTRs in everyday clinical practice [9].

Our study has the strength of the use of one of the more investigated noninvasive imaging methods for measuring liver steatosis and fibrosis. In addition, CAP measurement was assessed by using both FibroScan probes (M and XL). However, our study has few limitations. For the first, cross-sectional design of this study precludes any causal inferences about the directionality of the connections investigated in our study, its dynamics in time, and effects of graft and recipient outcomes. Second, we have used TE with CAP, instead of LB. This makes it impossible to evaluate the initial finding of steatosis in the graft and its dynamics on the posttransplantation finding. However, LB is an invasive procedure, and in our transplant center, we do not perform protocol biopsies. Instead of LB, we have used TE with CAP that is the best investigated and validated noninvasive imaging elastographic method for steatosis and fibrosis detection and quantification. Finally, CAP and LSM are not investigated in the post-LT setting, and we do not know the optimal cutoff values of CAP and LSM for each steatosis and fibrosis stage. However, in our population of LTRs, we have shown that metabolic risk factors (i.e., Mets and its individual components) are associated with CAP measurements, as well as they are associated in the pretransplant setting.

In conclusion, in our study, the prevalence of increased CAP values (i.e., steatosis) was 68.57%, while the prevalence of severe liver steatosis was 46.85%. Moreover, more than half of our LTRs had moderate elevation of LSM (i.e., fibrosis), while the prevalence of advanced liver fibrosis was 24%. Metabolic syndrome components were highly present in our cohort of patients (as well as in other studies) and were associated with CAP and LSM values as well as in the pretransplant setting. Regarding the earlier observations and our result about the high prevalence of metabolic comorbidities and NAFLD after LT and the lack of the abnormal liver test in a significant number of these patients, we strongly believe that TE with CAP may be a reasonable initial assessment for LTRs patients with one or more components of the MetS. As LTRs are living longer post-LT, it is important to investigate the long-term impact of NAFLD on survival of this population of patients [5–7]. Also, it is important to investigate the relationship of NAFLD with CVD and CKD morbidity and mortality in the post-LT setting. In the future, the investigations with protocol biopsies will have to analyze whether CAP and LSM as a surrogate marker of steatosis and fibrosis can be used in prediction of clinically relevant end points (liver related and nonliver related) in LTRs.

Data Availability

The data used to support the findings of this study are not available due to ethical restrictions.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors contributed equally to this review. All authors have read and agreed to the published version of the manuscript.

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Research Article

Metabolic and Hepatic Effects of Energy-Reduced Anti-Inflammatory Diet in Younger Adults with Obesity

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Background. Associated with epidemics of obesity, nonalcoholic fatty liver disease (NAFLD) is becoming the most prevalent liver disease worldwide. The cornerstone of therapy for NAFLD is lifestyle intervention, mainly focused on weight loss. Significant weight loss results from energy-restricted diets, regardless of macronutrient distribution. An anti-inflammatory diet was related to lower odds of NAFLD among daily alcohol drinkers and individuals with metabolic syndrome. This study aims to evaluate the effect of an energy-reduced anti-inflammatory diet on liver status in younger adults with obesity after a 6-month follow-up. *Methods*. A two-arm randomized controlled trial surveyed 81 participants' (mean age, 43 years) anthropometric and body composition changes. Metabolic status was determined with glycaemic and lipid status, inflammatory status with hs-CRP, IL-6, and TNF-α, and liver status with liver enzymes, NAFLD-FLS, FLI, and FIB-4 indices. The inflammatory potential of the diet was assessed by the Dietary Inflammatory Index, DII®. *Results*. Energy-restricted anti-inflammatory diet resulted in significant weight loss (−7.1%, $p < 0.001$), in reducing the visceral adiposity (−22.3%, $p < 0.001$), metabolic (HOMA-IR, −15.5%; total cholesterol, −5.3%; LDL-C, −4.6%; triglycerides, −12.2%), and inflammatory biomarkers (hs-CRP, −29.5%; IL-6, −18.2%; TNF-α, −34.2%), with significant improvement of liver parameters (NAFLD-FLS, −143.4%; FLI, −14.3%; FIB-4, −2.5%). *Conclusion*. The study showed the effectiveness of the anti-inflammatory diet with significant improvement of liver parameters in younger adults with obesity, which may reinforce the effectiveness of nutrition-based lifestyle programs, with an anti-inflammatory dietary approach for the treatment and resolution of NAFLD.

1. Introduction

In obesity, the accumulation of fat in the liver is associated with insulin resistance and subacute liver inflammation [1,2]. The most common subtype of liver fat accumulation is a nonalcoholic fatty liver disease (NAFLD), which progresses in individuals without excessive alcohol consumption, strong genetic predispositions, or use of steatogenic medication [3]. It was suggested that NAFLD is a risk factor for cardiovascular diseases and extrahepatic cancers, because NAFLD can potentially progress into nonalcoholic steatohepatitis and the later into cirrhosis and hepatocellular carcinoma [4]. Due to the obesity multisystem effect, the prevalence of NAFLD goes associated with the prevalence of obesity, making the most serious health threat responsible for increasing the number of cardiovascular, oncologic, and liver-related morbidity and mortality [5]. The burden of obesity-associated NAFLD can be ameliorated with lifestyle interventions, mainly by inducing weight loss and maintain a healthy body weight [6]. Short-term energy intake restriction resulted in a reduction in intrahepatic triglyceride storage [7,8], but the metabolic and hepatic effects of such lifestyle changes are less well understood [9]. To improve liver steatosis, 3%–5% loss in body weight is recommended, with greater liver status improvements when the weight loss is higher [10,11]. Marin-Alejandre et al. [12] showed that higher adherence to the Mediterranean diet resulted in a greater reduction in body weight, total fat mass, and hepatic fat and suggested additional benefits to weight loss in the treatment of obesity and associated comorbidities, such as NAFLD. However, the effects of dietary components, characteristics, and strategies for NAFLD treatment require more research [12–14]. The growing body of scientific evidence suggests that diet and dietary components are involved in the path of inflammation and consequently the pathogenesis of NAFLD. A diet with higher proinflammatory potential has been shown to be associated with higher odds for NAFLD development [15,16]. According to ATTICA study results, an anti-inflammatory diet was related to lower odds of NAFLD among daily alcohol drinkers and individuals with metabolic syndrome [17]. The PREDIMED substudy [16] reinforced the concept that obesity is associated with liver damage and revealed that the consumption of a proinflammatory dietary pattern might contribute to obesity and fatty liver disease features. The authors suggested that a well-designed precision diet containing acknowledged anti-inflammatory dietary components could specifically prevent and ameliorate obesity-related nonalcoholic fatty liver manifestations [16].

In this study, we present the changes in metabolic and hepatic parameters achieved with an energy-reduced anti-inflammatory diet among younger adults with obesity, with or without obesity-related complications.

2. Participants and Methods

2.1. Participants. The participants were recruited during their first visit to the obesity outpatient clinic at the Clinical Hospital Centre Rijeka, Croatia. The inclusion criteria were an age of 18 to 50 years, BMI $\geq 30\,kg/m^2$ with or without obesity-related complications, and stable body weight for the previous three months. Exclusion criteria were cigarette smoking within 6 months before study initiation, chronic heart, kidney, and/or severe liver disease, malignant disease or history of malignant disease, use of anti-inflammatory or immunosuppressive drugs or medications for weight loss, changes in chronic medications, active infection or surgical procedure in the previous three months, food allergy or intolerance to any anti-inflammatory diet constituent, pregnancy, and lactation.

2.2. Study Protocol. This six-month two-arm randomized controlled trial was designed to compare the effects of two dietary plans for weight loss with different nutritional characteristics on body weight, body composition, and metabolic, hepatic, and inflammation statuses in young adults with obesity. After the study presentation and baseline assessments, the recruited participants were randomly assigned to the anti-inflammatory diet (AID) group or the control diet (CD) group using a web-based randomization system (https://www.random.org/), administrated by trained medical personnel not engaged in any other study procedure. The study was conducted between March and October 2019 at Clinical Hospital Centre Rijeka, Croatia, previously approved by the ethics committee of the Clinical Hospital Centre Rijeka (Reg. No: 2170-29-02/15-16-4, January 31st, 2017) and conducted in line with the Declaration of Helsinki. All of the participants provided written informed consent before participating in the study. The study protocol has been registered with clinicaltrials.gov: NCT03987776 and has been described in detail elsewhere [18]. After the randomization of the participants, a comprehensive assessment was carried out at the baseline and the endpoint of the study, including anthropometric measurements, body composition, biochemical, and dietary assessments. The questionnaire used in this study contained standard sociodemographic information, physical activity level, dietary habits, medications, dietary supplements use, and self-reported stress. Except for demographics, the questionnaire was repeated at the study end. The flowchart of the participants is shown in Figure 1.

2.3. Dietary Intervention. At the educational workshops, held each month by a clinical dietitian, the AID group participants were instructed and strongly encouraged to follow an energy-restricted diet, based on low glycaemic foods, whole-grain products, legumes, colourful vegetables and fruits, nuts, seeds, marine fish, olive oil, green/black tea, and multiple spices and herbs. The CD group participants were instructed and strongly encouraged to follow an isocaloric standard diet protocol for bodyweight reduction (55–60% carbohydrates, 25–30% fat, and 15–20% protein) [19]. Each dietary intervention has been described in a study protocol [18]. The AID group participant used more often olive oil, colourful low glycaemic index vegetables and fruits, nuts, seeds, onion, garlic, various spices, marine fish, and fermented dairy products and avoided red and processed

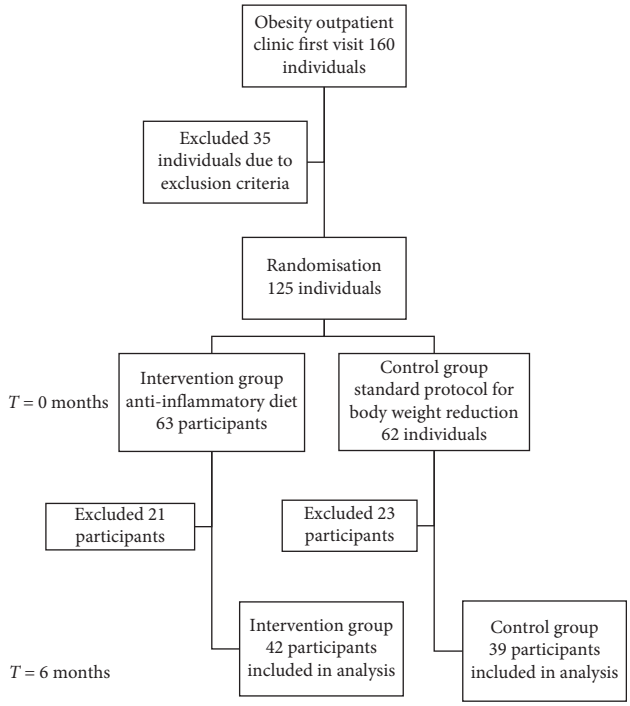

Figure 1: The flowchart of participants in the study trial.

meat and industrially processed foods to overcome an overlap in recommended daily intake of vegetables, fruits, legumes, whole grains, nuts, green tea, and herbs among the CD group. Daily resting energy expenditure was calculated for each participant according to Mifflin-St. Jeor's equation [20] using their baseline anthropometric measurements and then multiplied with the activity factor based on information from the physical activity questionnaire [21]. The value obtained from these equations was reduced by 25%, thus providing the recommended energy intake for each participant. The adjustments of the number and quantity of servings of each food group were made accordingly. At each workshop, meal planning with recipes, food serving sizes, specific food consumption, and personal goal-setting was discussed. Participants who had missed the educational workshop were provided with workshop materials.

The compliance with given dietary recommendations was monitored with 3-day food intake records (covering two weekdays and one weekend day) that each participant was asked to fulfil before a monthly group meeting (overall six 3-day food intake records). The dietary records results were discussed with each participant, and those whose dietary intervention adherence was less than 75% were considered as noncompliant and withdrawn from the trial. The baseline dietary habits that were assessed with a 133-item food frequency questionnaire (FFQ) [22] were discussed with each participant to correctly follow the dietary intervention. A Croatian food composition database [23] was used to calculate the energy and dietary components intake, and certain nutrients such as caffeine, β-carotene, omega-3, and omega-6 fatty acids intake were calculated using Danish [24] and American food composition database [25], the Phenol-Explorer 3.0 database [26], and USDA database [27]. The contents of the various polyphenols were multiplied by their retention factors, due to meal thermal processing [28].

The assessment of the inflammatory potential of the diet was done with the Dietary inflammatory index, DII® [29], which included all of its 45 parameters. For DII® calculation, firstly we calculated a z-score by adjusting each participant's dietary intake data against a reference global daily mean and standard deviation (SD) intake for each parameter. The global dietary intake data were based on consumption data from 11 countries [29]. For decreasing the effect of right-skewing of the dietary data, the z-score was expressed as a proportion (i.e., with the value from 0 to 1). The centring of provided scores on 0 was achieved by doubling the proportion and subtracting 1. The resulting centred proportion score for each dietary parameter was multiplied by its respective parameter-specific inflammatory effect score and then each calculated 45 scores were summed to achieve an overall DII score of each participant [29]. The positive DII® score values specified a proinflammatory diet, and negative values an anti-inflammatory diet [29]. The dietary data were provided from the food frequency questionnaire (FFQ) [18] at the study baseline and its end, for obtaining the intake frequency (from once per month to a few times per day) and food and beverage portion size (small, medium, and large) information. To the standard list of 97 food items that were represented in the FFQ, for this trial, we added 36 food items and herbs and spices with anti-inflammatory properties.

2.4. Anthropometric, Body Composition, and Biochemical Assessment. The assessment of anthropometric measurements (body weight, height, and waist circumference), body composition by the bioelectrical impedance method (Seca mBCA 515, Seca gmbh and co. Kg, Hamburg, Germany), and blood pressure (Omron® HEM 705 CP, Health-care Co, Kyoto, Japan) was carried out under fasting conditions at the obesity outpatient clinic at the Clinical Hospital Centre Rijeka, Croatia following standardized procedures, as previously described [18]. Body Mass Index (BMI) was calculated as the bodyweight divided by the squared height (kg/m^2). Biochemical assessments, including concentrations of blood glucose, glycated haemoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and high sensitivity C-reactive protein (hs-CRP) were measured on an Olympus 5800 (Olympus) with specific commercial kits. Insulin was analysed with the CLIA method on Immulite 2000xp, Siemens. The ELISA method was used for the measurement of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) with assay kits purchased from eBioscience™ (Thermo Fisher Scientific, Waltham, USA).

The insulin resistance was assessed using the Homeostasis Model Assessment Index (HOMA-IR) [30]. The metabolic syndrome was assessed by the presence of three or more parameters according to the definition by the International Diabetes Federation Task Force on Epidemiology and Prevention [31].

Currently, the "golden" standard for NAFLD diagnosis is liver biopsy, but it is invasive, in some cases clinically unavailable, also time and money consuming. The use of blood biomarkers and particular indices for NAFLD diagnosis may be useful to select individuals who need NAFLD ultrasonography screening as a noninvasive tool for assessing fibrosis and making the decision of whether to perform a liver biopsy. It was shown that the vast majority of patients will never develop severe liver disease, so it is neither realistic nor necessary to perform a liver biopsy in all patients [5]. Therefore, for estimation of liver fat content in NAFLD, i.e., hepatic steatosis, we used NAFLD-FLS score and modified Fatty Liver Index, and for estimating the liver fibrosis possibility, we used Fibrosis Index based on four factors (FIB-4 index).

NAFLD-FLS score [32,33] was assessed according to the formula: NAFLD-FLS = − 2.89 + 1.18 × MetS (yes: 1; no: 0) + 0.45 × diabetes mellitus (yes: 2; no: 0) + 0.15 × insulin (mU/L) + 0.04 × AST (U/L) − 0.94 × AST/ALT. We used a NAFLD-FLS cutoff of > −0.64 to classify those with hepatic steatosis.

A modified Fatty Liver Index (FLI) [34,35] was assessed according to the formula: liver fat (%) = 10(−0.805 + 0.282 ∗ metabolic syndrome (yes = 1; no = 0) + 0.078 ∗ type 2 diabetes (yes = 1; no = 0) + 0.525 LOG(fS−insulin (mU/L) + 0.521 ∗ LOG(fS−AST (U/L) − 0.454 ∗ LOG (AST/ALT), with a cutoff of >0.8 for classifying those with hepatic steatosis.

FIB-4 index [36] was assessed according to the following formula: FIB-4 = (age × AST)/[PLT($\times 10^9$/L) × ($\sqrt{}$ALT)], with a cut off of >1.45 for classifying the possibility of liver fibrosis.

2.5. Statistical Analyses. The statistically significant sample size for this study was estimated using the data from a recent randomized controlled trial that compared the effects of two dietary strategies for weight loss with different nutritional characteristics among subjects with obesity and NAFLD [12]. With a 95% confidence interval ($\alpha = 0.05$) and a statistical power of 90% ($\beta = 0.9$), group size ratio 1 : 1, and using t test for repeated measures, it was calculated that 42 participants per group were needed, but considering the estimated dropout rate of 25%, at least 53 participants per each study group were considered for the study inclusion.

The mean value (standard deviation) described the studied variables. The evaluated variables were tested for normality of the distribution by the Kolmogorov-Smirnov test. The differences between the study groups were compared with Student's t test or the Mann–Whitney U test when appropriate. The differences between the beginning and the end of the intervention period within each group were analysed by a paired Student's t test or Wilcoxon test when appropriate. Categorical variables were compared using a chi-squared test. All parameters' changes were calculated with z-score ((mean after intervention-baseline mean)/baseline mean ×100). Linear regression analyses were used to evaluate the potential association between the anthropometry, body composition, metabolic, inflammatory, and hepatic status variables with the inflammatory potential of the diet, with adjustments for age, sex, educational level, physical activity, and obesity degree. All tests were performed with Statistica 12.7 for Windows (Statsoft Inc, Tulsa, OK, SAD), which were regarded as 2-tailed, and p values <0.05 were considered as statistically significant.

3. Results

After 6 months of nutritional intervention, out of 125 participants fulfilling inclusion and exclusion criteria, 63 were randomized to the AID group and 62 to the CD group. A total of 81 participants (42 in the AID group and 39 in the CD group) completed the trial evaluation and entered in all trial calculations. Noncompliance with the dietary recommendations was the main reason for exclusion with dropouts that were similar for both groups. The flowchart of participants has been shown in Figure 1. The majority of trial participants in both groups were female (93%, $p < 0.001$ vs 90%, $p < 0.001$) (Table 1). There were almost half of the participants with three or more components of the metabolic syndrome in the AID group. At the end of the trial, the proportion of participants fulfilling the criteria for metabolic syndrome decreased by almost half in the AID group ($p = 0.042$) and by 30% in the CD group ($p = 0.314$). The number of participants with hyperglycaemia as assessed with HbA1c values higher than 6.5 reduced in half after the 6 months of the trial in the AID group, but not significantly ($p = 0.057$), while it significantly reduced in the CD group ($p = 0.003$). In the AID group, hepatic steatosis assessed with NAFLD-FLS and with FLI was detected in 43% and 48% of participants, respectively, and both reduced in half at the trial end, but not significantly. In the CD group, hepatic steatosis assessed with NAFLD-FLS was detected at 38% of participants. That proportion reduced significantly for third (p = 0.019), and while assessed with FLI, it reduced by 14%, but not significantly. . The possibility for liver fibrosis had around 5% of participants in both dietary groups and significantly reduced to 0% at the trial end $p < 0.001$ and $p < 0.001$, respectively).

A significant weight loss has been achieved in both dietary groups (−7.1%, $p < 0.001$ vs −6.2%, $p < 0.001$) (Table 1). Also, BMI, total body fat, and visceral fat decreased significantly in both dietary groups, while the proportion of nonfat tissue significantly increased. No statistically significant differences were found between the intervention groups for the mentioned variables nor at the baseline nor the trial end (Table 1).

Both dietary groups showed improvements in glycaemic, lipid, and inflammatory parameters. Fasting glucose, insulin, and HOMA-IR were reduced in both groups; however, these changes were statistically significant only in the CD group (Table 1). Moreover, the CD group achieved significant reductions in the total and LDL-cholesterol concentrations ($p = 0.002$ and $p < 0.001$, respectively) (Table 1). Biomarkers of inflammation were significantly reduced in both groups. The AID group participants achieved greater reduction in TNF-α (−34.2%, $p = 0.002$ vs −10.5%, $p = 0.001$,

Table 1: Patient characteristics and changes in anthropometric and biochemical parameters at baseline and after 6 months of dietary intervention.

Variable	Anti-inflammatory diet group ($n = 42$)				Control diet group ($n = 39$)				Baseline p-value[b]	6-months p-value[c]
	Baseline	6 months	Change (%)	p-value[a]	Baseline	6 months	Change (%)	p-value[a]		
Sex (men/women)	3/39		–	<0.001[d]	4/35		–	<0.001[d]	0.619[d]	–
Metabolic syndrome (yes)	20	11	−45.0	0.042[d]	13	9	−30.8	0.314[d]	0.191[d]	0.746[d]
HbA1c ≥ 6.5%	12	5	−58.3	0.057[d]	18	6	−66.7	0.003[d]	0.102[d]	0.648[d]
NAFLD-FLS > −0.64	18	13	−27.8	0.062[d]	15	10	−33.3	0.019[d]	0.916[d]	0.596[d]
FLI > 0.8	20	15	−25.0	0.184[d]	21	18	−14.3	0.749[d]	0.575[d]	0.339[d]
FIB-4 > 1.45	2	0	−100.0	<0.001[d]	2	0	−100.0	<0.001[d]	0.938[d]	0.992[d]
Age (years)	43.6 (5.8)		–	–	41.7 (6.7)		–	–	0.178	–
Anthropometry and body composition										
Body weight (kg)	102.9 (14.2)	95.7 (11.7)	−7.1	<0.001	101.4 (21.9)	95.1 (21.4)	−6.2	<0.001	0.770	0.903
Body Mass Index (kg/m^2)	35.4 (4.3)	32.9 (3.9)	−7.0	<0.001	33.4 (5.5)	31.0 (4.3)	−7.2	<0.001	0.179	0.119
Waist circumference (cm)	108.4 (8.4)	102.9 (7.8)	−5.1	<0.001	107.9 (10.1)	100.9 (10.0)	−6.5	<0.001	0.482	0.442
Total fat tissue (%)	44.9 (4.4)	42.3 (4.8)	−5.6	<0.001	45.6 (2.6)	42.2 (3.0)	−7.4	<0.001	0.505	0.755
Visceral adipose tissue (*l*)	3.1 (1.3)	2.4 (1.0)	−22.3	<0.001	3.5 (1.6)	2.6 (1.4)	−25.4	<0.001	0.376	0.798
Nonfat tissue (%)	55.1 (4.4)	57.8 (4.7)	4.8	<0.001	54.4 (2.6)	57.2 (2.4)	5.2	<0.001	0.484	0.587
Skeletal muscle tissue (kg)	27.4 (3.9)	26.2 (3.3)	−4.3	0.022	27.0 (6.2)	25.8 (7.3)	−4.4	0.005	0.449	0.085
Biochemical parameters										
Glucose (mmol/l)	5.7 (1.4)	5.5 (0.6)	−3.7	0.284	5.6 (0.5)	4.9 (0.6)	−13.1	<0.001	0.107	0.001
HbA1c (mmol/mol)	35.3 (6.5)	34.7 (7.6)	−1.7	0.855	38.3 (4.9)	38.4 (4.7)	0.1	0.121	0.128	0.050
Insulin (mU/l)	18.2 (11.7)	16.2 (10.0)	−11.1	0.946	16.1 (4.9)	11.7 (3.9)	−27.1	0.008	0.419	0.048
HOMA-IR (pmol/l)	4.8 (3.9)	4.1 (3.0)	−15.5	0.307	4.0 (1.3)	2.5 (0.9)	−36.3	0.002	0.572	0.040
Total cholesterol (mmol/l)	5.3 (1.1)	5.0 (1.34)	−5.3	0.594	5.8 (0.7)	5.4 (0.8)	−7.7	0.002	0.028	0.193
HDL-C (mmol/l)	1.4 (0.2)	1.5 (0.53)	10.2	0.058	1.3 (0.2)	1.3 (0.1)	−0.8	0.073	0.642	0.127
LDL-C (mmol/l)	3.3 (1.0)	3.2 (0.99)	−4.6	0.354	3.8 (0.6	3.4 (0.6)	−12.0	<0.001	0.031	0.343
Triglycerides (mmol/l)	1.3 (0.9)	1.2(0.56)	−12.2	0.445	1.5 (0.4)	1.3 (0.5)	−11.3	0.393	0.008	0.144
Platelet (×10^9/l)	261.3 (73.4)	248.3 (77.1)	−5.0	0.289	290.9 (104.3)	286.9 (85.3)	−1.4	0.226	0.268	0.049
hs-CRP (mg/l)	6.3 (5.5)	4.4 (4.29)	−29.5	0.003	6.8 (4.1)	3.9 (0.9)	−42.2	0.010	0.311	0.662
IL-6 (pg/mL)	0.8 (0.6)	0.6 (0.36)	−18.2	0.013	1.3 (0.9)	1.0 (0.8)	−26.9	0.002	<0.001	0.001
TNF-α (pg/mL)	0.4 (0.2)	0.3 (0.09)	−34.2	0.002	1.7 (0.3)	1.5 (0.4)	−10.5	<0.001	0.001	<0.001

Data are presented as number or the mean (SD). NAFLD-FLS, Nonalcoholic Fatty Liver Disease Liver Fat Score; FLI, Fatty Liver Index; FIB-4, Fibrosis Index based on four factors; HbA1c, glycated haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; hs-CRP: high sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha. [a]Comparison within dietary groups (baseline and after 6 months). [b]Baseline differences between the AID and CD groups. [c]Differences after 6 months between the AID and CD groups. [d]Chi-squared test for baseline differences between the AID and CD groups.

respectively), while the CD group participants reduced hs-CRP (29.5%, $p = 0.003$ vs −42.12%, $p = 0.010$, respectively) and IL-6 concentrations (−18.2%, $p = 0.013$ vs −26.9%, $p = 0.002$, respectively) slightly more than the AID group participants. Only the changes in glycaemic parameters ($p = 0.001$, $p = 0.050$, $p = 0.048$, $p = 0.040$, respectively), IL-6 ($p = 0.001$), and TNF-α ($p = 0.001$) from the baseline to 6 months of intervention differed significantly between the dietary groups.

A reduction in liver enzymes (AST, ALT, and GGT) was observed in both groups; however, these changes were statistically significant only for GGT (−21.3%, $p = 0.011$ in the AID group and −14.3%, $p = 0.003$ in the CD group) (Table 2). A significant reduction in the Fatty Liver Index was achieved with both dietary interventions ($p = 0.040$ and $p = 0.006$, respectively). NAFLD-FLS and FIB-4 indices notably reduced in both groups but not significantly (Table 2). Only the changes in GGT ($p = 0.040$) and FLI ($p = 0.047$) from the

Table 2: Liver parameters at baseline and after 6 months of dietary intervention.

Variable	Anti-inflammatory diet group ($n = 42$)				Control diet group ($n = 39$)				Baseline p value[b]	6 months p value[c]
	Baseline	6 months	Change (%)	p value[a]	Baseline	6 months	Change (%)	p value[a]		
AST (IU/L)	21.7 (7.9)	20.7 (6.1)	−4.8	0.516	24.0 (6.5)	23.0 (6.4)	−4.2	0.885	0.263	0.075
ALT (IU/L)	24.3 (13.5)	22.6 (11.2)	−6.8	0.914	31.9 (13.1)	29.3 (14.7)	−8.1	0.416	0.540	0.099
GGT (IU/L)	22.43 (9.9)	17.7 (6.7)	−21.3	0.011	25.4 (5.6)	21.8 (6.6)	−14.3	0.003	0.212	0.040
NAFLD-FLS	0.46 (2.2)	−0.2 (2.1)	−143.4	0.158	0.0 (0.8)	−0.1 (1.4)	−275.0	0.590	0.647	0.875
FLI	1.4 (0.6)	1.2 (0.5)	−14.3	0.040	1.6 (0.7)	1.3 (0.7)	−18.8	0.006	0.331	0.047
FIB-4	0.8 (0.2)	0.8 (0.2)	−2.5	0.452	1.2 (2.0)	0.7 (0.3)	−41.7	0.207	0.418	0.495

Data are presented as the mean (SD). AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; NAFLD-FLS, Nonalcoholic Fatty Liver Disease Liver Fat Score; FLI, Fatty Liver Index; FIB-4, Fibrosis Index Based On Four Factors. [a]Comparison within dietary groups (baseline and after 6 months). [b]Baseline differences between the AID and CD groups. [c]Differences after 6 months between the AID and CD groups.

baseline to 6 months of intervention differed statistically significant between the two dietary strategies.

Furthermore, there were no significant differences at baseline concerning dietary intake, except for higher intake of MUFA ($p = 0.018$), omega-3 fatty acids ($p = 0.010$), total polyphenols ($p = 0.002$), and a lower intake of dietary cholesterol ($p = 0.004$) by the AID group (Table 3). Regarding changes from baseline to 6 months of intervention, both dietary groups achieved a significant reduction in energy intake ($p < 0.001$) and saturated fat energy share ($p < 0.001$). Both groups significantly raised the proportion of total energy intake from proteins ($p < 0.001$) and MUFA ($p < 0.001$), intake of fibre ($p < 0.001$), and total polyphenols ($p = 0.019$ and $p < 0.001$, respectively). The AID group significantly reduced the proportion of total energy intake from carbohydrates ($p < 0.001$) and dietary cholesterol intake ($p = 0.030$) and significantly increased the proportion of total energy intake from total fat ($p = 0.021$), PUFA ($p = 0.029$), and omega-3 fatty acids ($p < 0.001$). The CD group significantly reduced the proportion of total energy intake from alcohol ($p = 0.037$). Both dietary groups significantly raised the intake of flavones ($p < 0.001$ and $p = 0.037$, respectively) and reduced the intake of flavonones ($p < 0.001$, $p < 0.001$, respectively), which the CD group reduced significantly more than AID group ($p = 0.002$, $p < 0.001$, respectively). The intakes of other flavonoid subgroups were raised by both dietary groups but not significantly. As expected, the AID group significantly decreased the DII® value ($p = 0.002$), significantly more than the CD group ($p < 0.001$).

Linear regression analyses (adjusted by a group of intervention, age, sex, physical activity, medication use, and obesity comorbidities) were performed to evaluate the anthropometric, biochemical, and dietary factors potentially involved with liver parameters after the 6 months of the dietary intervention (Tables 4 and 5). Models were not adjusted for total dietary energy and dietary supplements intake because they are the DII® components. We noticed that the weight loss and reduction of BMI and visceral fat tissue were associated with improvements in hepatic status but not significantly (Tables 4 and 5). The decrease in total fat tissue was significantly associated with a reduction in Fatty Liver Index ($p = 0.037$) and Fibrosis Index based on four factors in the CD group after adjustment ($p = 0.021$) (Table 5). Regarding inflammatory markers, we found that their reduction was associated with improvements in hepatic status, but not significantly, except for IL-6 with FLI in the CD group after adjustments ($p = 0.020$). Concerning dietary factors, the decrease of DII® and energy was significantly associated with the decrease of FIB-4 index in the AID group ($p = 0.044$ and $p = 0.042$, respectively). Also, in the same dietary group, the increase in total dietary fat influenced the FIB-4 index increase after the adjustment ($p = 0.031$). At the same time, we found that, among the AID group, the decrease of flavones and flavonones was associated with improvement in FIB-4 ($p = 0.043$ and $p = 0.047$, respectively) and of flavonols with FLI ($p = 0.048$) after adjustment (Table 4). After adjustment, it was found that the decrease in flavones and in anthocyanidins resulted in significant improvements of FLI in the CD group ($p = 0.027$ and $p = 0.012$, respectively). The increase in protein intake resulted in improvements in FLI ($p = 0.043$) among CD group participants after adjustment (Table 5).

4. Discussion

The present randomized controlled trial that compared the effects of two energy-restricted dietary interventions on anthropometry, body composition, and biochemical parameters and the non-invasive parameters of liver status in younger adults with obesity resulted in noteworthy improvements in liver enzymes, and in hepatic status indices. Both dietary groups achieved significant improvements in their anthropometric and body composition parameters, with no significant difference between them after the 6 months of the trial. Participants that consumed an energy-reduced anti-inflammatory diet achieved a greater reduction in total body weight, while participants in the CD group obtained slightly larger reduction of total fat tissue and visceral adipose tissue associated with improvement in FLI and FIB-4. The AID group achieved a more significant reduction of GGT and similar of FLI. An important

Table 3: Dietary intake at baseline and after 6 months of dietary intervention.

Variable	Anti-inflammatory diet group ($n = 42$)				Control diet group ($n = 39$)				Baseline p value[b]	6-months p value[c]
	Baseline	6 months	Change (%)	p value[a]	Baseline	6 months	Change (%)	p value[a]		
Energy (MJ)	10.0 (2.6)	6.9 (0.5)	−31.0	<0.001	11.2 (2.6)	7.6 (0.4)	−31.9	<0.001	0.129	<0.001
Protein (%MJ)	17.2 (1.7)	20.6 (2.6)	20.2	<0.001	17.1 (2.0)	21.3 (1.9)	25.0	<0.001	0.872	0.292
Carbohydrate (% MJ)	38.6 (6.1)	35.3 (7.7)	−8.6	<0.001	41.6 (4.7)	38.0 (3.7)	−8.7	0.535	0.063	0.131
Total fat (%MJ)	42.6 (6.5)	44.0 (6.2)	3.2	0.021	40.3 (3.7)	39.8 (3.7)	−1.3	0.005	0.133	0.006
MUFA (%MJ)	17.3 (4.5)	21.4 (6.9)	26.8	<0.001	14.9 (1.8)	16.0 (3.5)	8.3	0.856	0.018	<0.001
PUFA (%MJ)	7.3 (2.1)	8.4 (2.9)	17.4	0.029	7.1 (0.8)	6.5 (1.2)	−7.8	0.001	0.677	<0.001
Omega-3 (%MJ)	0.5 (0.3)	0.7 (0.4)	56.8	<0.001	0.3 (0.1)	0.3 (0.1)	0.0	0.109	0.010	<0.001
Omega-6 (%MJ)	0.3 (0.1)	0.3 (0.2)	−3.7	0.003	0.3 (0.1)	0.3 (0.1)	−3.2	0.159	0.210	0.071
Saturated fat (%MJ)	15.6 (2.9)	11.0 (24.6)	−29.5	<0.001	16.6 (2.5)	14.1 (33.8)	−14.8	<0.001	0.199	<0.001
Trans fat (%MJ)	0.7 (0.3)	0.7 (0.5)	14.3	0.279	0.7 (0.2)	0.7 (0.3)	−9.7	0.214	0.363	0.363
Cholesterol (mg)	380.8 (160.4)	318.5 (175.6)	−16.4	0.030	477.6 (463.0)	463.0 (125.6)	−3.0	0.643	0.004	<0.001
Fiber (g)	27.4 (11.3)	33.9 (5.2)	23.5	0.002	25.5 (6.7)	28.7 (3.9)	12.7	0.146	0.467	<0.001
Alcohol (%MJ)	1.6 (2.8)	1.6 (2.8)	0.0	0.999	1.1 (1.3)	0.9 (1.3)	−12.7	0.006	0.464	0.037
Total polyphenols (mg)	688.4 (240.0)	733.7 (106.0)	6.6	0.019	472.7 (200.8)	740.6 (98.3)	56.7	<0.001	0.002	0.817
Flavan-3-ol (mg)	28.8 (26.8)	15.8 (10.4)	−45.1	0.056[b]	23.4 (12.8)	8.3 (2.4)	−64.3	<0.001[a]	0.807[b]	<0.001[b]
Flavones (mg)	2.9 (2.03)	5.6 (3.4)	92.4	<0.001[a]	2.3 (1.4)	3.1 (1.5)	38.3	0.037[b]	0.366[b]	0.002[a]
Flavonols (mg)	147.4 (78.6)	149.4 (6.8)	1.4	0.856[a]	107.6 (34.8)	74.2 (40.0)	−31.0	<0.001[a]	0.029[a]	<0.001[a]
Flavonones (mg)	46.1 (37.9)	23.0 (22.9)	−50.2	<0.001[a]	65.6 (74.7)	3.6 (4.0)	−94.5	<0.001[a]	0.856[b]	<0.001[b]
Anthocyanidins (mg)	24.3 (34.6)	30.0 (28.4)	23.6	0.406[a]	15.5 (9.3)	24.3 (19.4)	56.4	0.606[a]	0.873[b]	0.426[a]
Isoflavones (mg)	0.4 (0.2)	0.6 (0.3)	55.6	0.864[a]	0.3 (0.4)	0.6 (0.5)	129.6	0.787[a]	0.816[a]	0.374[a]
DII®	−0.5 (2.3)	−2.0 (1.0)	283.0	0.002	−0.2 (1.3)	−0.3 (1.0)	30.4	0.725	0.579	<0.001

Data are presented as the mean (SD). DII®, Dietary Inflammatory Index. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [a]Comparison within dietary groups (baseline and after 6 months). [b]Baseline differences between the AID and CD groups. [c]Differences after 6 months between the AID and CD groups.

Table 4: Regression analyses of the liver parameters after 6 months of dietary intervention as dependent variables and changes in anthropometric, biochemical, and dietary factors as independent variables in the AID group.[a]

Variable changes (Δ)	Anti-inflammatory diet group											
	NAFLD-FLS				FLI				FIB-4			
	Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
	β	p value	β	p value	β	p value	β	p value	β	p value	β	p value
Bodyweight (kg)	−0.47	0.418	−1.00	0.280	−0.43	0.379	0.75	0.102	−0.70	0.381	−1.41	0.089
BMI (kg/m^2)	−0.49	0.739	−1.95	0.291	−0.79	0.530	−1.44	0.111	−1.75	0.401	−1.59	0.222
Fat tissue (%)	−0.39	0.098	−0.21	0.282	−0.09	0.582	−0.09	0.265	−0.11	0.672	−0.35	0.070
Visceral adipose tissue (l)	−0.69	0.300	−0.53	0.324	−0.35	0.511	−0.21	0.308	−0.56	0.517	−0.80	0.097
hs-CRP (mg/l)	−0.16	0.120	−0.06	0.489	0.01	0.849	0.06	0.161	−0.18	0.192	−0.01	0.884
IL-6 (pg/mL)	0.15	0.985	−8.06	0.318	−5.14	0.462	−0.44	0.867	−1.71	0.876	−14.21	0.073
TNF-α (pg/mL)	−2.32	0.546	−9.16	0.131	−1.40	0.660	−2.68	0.199	−1.10	0.829	−9.68	0.059
DII®	−0.47	0.131	−1.28	0.225	−0.07	0.770	−1.10	0.060	−0.50	0.215	−2.27	0.044
Energy (MJ)	0.01	0.392	−0.01	0.204	−0.39	0.706	−0.01	0.067	0.01	0.436	−0.01	0.042
Proteins (%MJ)	−0.01	0.961	−0.21	0.198	−0.01	0.937	−0.11	0.118	0.10	0.522	−0.22	0.094
Total fat (%MJ)	−0.10	0.233	0.25	0.233	−0.05	0.467	0.19	0.081	0.01	0.930	0.55	0.031
Omega-3 (%MJ)	0.31	0.235	−0.32	0.394	0.16	0.458	−0.26	0.154	0.29	0.393	−0.69	0.074
Total polyphenols (mg)	−0.26	0.305	−0.18	0.543	0.01	0.586	0.01	0.070	0.01	0.470	0.01	0.099
Flavan-3-ol (mg)	0.05	0.862	−0.06	0.799	0.01	0.651	0.01	0.814	0.01	0.947	−0.01	0.436

Table 4: Continued.

Variable changes (Δ)	Anti-inflammatory diet group											
	NAFLD-FLS				FLI				FIB-4			
	Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
	β	p value	β	p value	β	p value	β	p value	β	p value	β	p value
Flavones (mg)	0.09	0.554	−0.40	0.211	−0.02	0.881	−0.33	0.061	0.08	0.709	−0.69	0.043
Flavonols (mg)	0.01	0.206	0.01	0.562	0.01	0.440	−0.01	0.048	0.01	0.598	−0.01	0.065
Flavonones (mg)	0.01	0.509	0.05	0.199	0.10	0.900	0.02	0.076	−0.01	0.682	−0.06	0.047
Anthocyanidins (mg)	−0.10	0.777	−0.05	0.159	0.01	0.695	−0.01	0.123	0.01	0.934	−0.03	0.053
Isoflavones (mg)	0.01	0.884	−0.02	0.294	0.01	0.755	0.01	0.180	0.01	0.286	0.01	0.125

[a]Models were adjusted by age, sex, physical activity, medication use, and obesity comorbidities. Models were not adjusted for total energy intake and dietary supplements because they are the DII® components. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; NAFLD-FLS, Nonalcoholic Fatty Liver Disease Liver Fat Score; FLI, Fatty Liver Index; FIB-4, Fibrosis Index based on four factors. DII®, Dietary Inflammatory Index; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha.

Table 5: Regression analyses of the hepatic status parameters after 6 months of dietary intervention as dependent variables and changes in anthropometric, biochemical, and dietary factors as independent variables in the CD group[a].

Variable changes (Δ)	Control diet group											
	NAFLD-FLS				FLI				FIB-4			
	Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
	B	p value	β	p value	β	p value	β	p value	β	p value	β	p value
Bodyweight (kg)	−0.05	0.819	−0.04	0.752	−0.03	0.605	0.03	0.599	0.10	0.750	−0.10	0.706
BMI (kg/m^2)	−0.02	0.969	0.01	0.967	−0.01	0.956	−0.11	0.313	0.67	0.324	0.36	0.481
Fat tissue (%)	−0.19	0.503	−0.15	0.126	−0.05	0.423	−0.09	0.037	−0.07	0.845	−0.53	0.021
Visceral adipose tissue (l)	−0.14	0.748	−0.10	0.620	−0.01	0.900	0.05	0.525	1.36	0.094	0.66	0.123
hs-CRP (mg/l)	−0.17	0.350	−0.16	0.081	−0.01	0.849	−0.07	0.058	0.07	0.777	0.07	0.639
IL-6 (pg/mL)	−0.60	0.614	−0.78	0.247	−0.25	0.504	−0.61	0.020	−2.36	0.313	−0.08	0.928
TNF-α (pg/mL)	−1.16	0.609	−0.94	0.425	−0.31	0.562	−0.69	0.172	−5.78	0.136	−2.97	0.213
DII®	0.01	0.967	0.01	0.991	0.06	0.484	0.10	0.360	0.02	0.965	0.16	0.749
Energy (MJ)	0.01	0.694	0.01	0.591	0.01	0.566	0.01	0.188	0.01	0.638	−0.03	0.921
Proteins (%MJ)	0.01	0.997	−0.02	0.891	0.05	0.414	0.12	0.043	−0.30	0.381	−0.13	0.565
Total fat (%MJ)	−0.01	0.944	−0.01	0.891	0.01	0.631	0.03	0.331	−0.21	0.186	−0.19	0.179
Omega-3 (%MJ)	7.98	0.431	7.27	0.142	0.77	0.734	4.03	0.059	−13.45	0.354	−6.38	0.460
Total polyphenols (mg)	0.02	0.298	0.02	0.167	0.01	0.816	0.02	0.975	−0.02	0.584	−0.01	0.660
Flavan-3-ol (mg)	0.02	0.708	0.02	0.613	0.01	0.480	0.02	0.181	−0.11	0.205	−0.05	0.402
Flavones (mg)	−0.40	0.147	−0.42	0.027	0.03	0.547	0.03	0.584	−0.84	0.064	−0.58	0.074
Flavonols (mg)	−0.03	0.297	−0.03	0.118	0.02	0.535	−0.01	0.346	0.03	0.366	0.00	0.862
Flavonones (mg)	0.01	0.370	0.00	0.234	0.01	0.410	0.00	0.413	0.00	0.711	0.00	0.727
Anthocyanidins (mg)	−0.07	0.148	−0.07	0.012	0.01	0.605	−0.02	0.059	0.03	0.585	−0.01	0.848
Isoflavones (mg)	0.05	0.930	0.01	0.891	0.21	0.204	0.18	0.188	−0.98	0.288	−0.05	0.402

[a]Models were adjusted by age, sex, physical activity, medication use, and obesity comorbidities. Models were not adjusted for total energy intake and dietary supplements because they are the DII® components. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; NAFLD-FLS, Nonalcoholic Fatty Liver Disease Liver Fat Score; FLI, Fatty Liver Index; FIB-4, Fibrosis Index based on four factors. DII®, Dietary Inflammatory Index; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.

contributing factor to adverse clinical outcomes, including NAFLD's pathophysiology, is excess body weight [37]. For that reason, weight loss management had been suggested as the most important factor for NAFLD treatment [37,38].

It was shown that weight loss of ≥3% was able to improve liver steatosis, although at least 5% weight loss is needed to improve inflammation and hepatic histology [39] and to stabilize fibrosis [40,41]. Besides, the weight loss of 7% or more resulted in improvement of nonalcoholic steatohepatitis (NASH) in 65–90% of patients [40–42]. In our study, both studied groups reached on average 7% loss of their baseline weight and achieved a significant reduction in the Fatty Liver Index and GGT level. A higher decrease in total adipose tissue was observed in the CD group which was significantly associated with lower liver fibrosis estimated with FIB-4 index. On the other hand, it was noticed that visceral adipose tissue reduction in the CD group was associated with improvements in liver steatosis and liver fibrosis after adjustments for potential confounders such as age, sex, physical activity, use of medications, and obesity comorbidities. The CD group had higher FIB-4 values at the baseline which perhaps did not notably reduce after 6 months of the trial in those who had a lower reduction of total and visceral adipose tissue, but after the adjustments,

the reduction of total fat tissue was significantly associated with its reduction. The reduction of total fat tissue among participants in the AID group has been associated with improvements in the NAFLD-FLS and FLI index and in liver fibrosis estimated with FIB-4 index but not significantly. It was shown that liver fibrosis progression does not occur in all patients with diagnosed NAFLD and not at the same rate [5], which is in line with the results of our study.

The distribution of body fat is a main pathophysiological mechanism for metabolic disease, where abdominal obesity differs from a more equally fat distribution [43]. Free fatty acids (FFAs) released from hypertrophic adipocytes, especially from visceral adipose tissue, induce systemic and hepatic insulin resistance which successively intensifies the release of FFAs from adipose tissue. Excessive amounts of circulating FFAs ultimately lead to hypertriglyceridemia and consequently NAFLD [44]. Furthermore, the accumulation of liver fat is strongly associated with diminishing adipose tissue insulin sensitivity [45]. NAFLD appears to increase the chances of developing nonfatal coronary heart disease, ischemic stroke, or cardiovascular death by more than 50% in patients with T2D [43]. In our study, 37% of participants had HbA1c $\geq$ 6.5% at baseline, indicating the diagnosis of type 2 diabetes. After the dietary intervention, this number decreased to only 14% of participants suggesting better glycaemic control or even diabetes remission in those not taking or eliminating diabetes medications. Although all participants in this trial improved their glycaemic status, the CD group participants significantly more improved their insulin resistance assessed with HOMA-IR. HOMA-IR in the AID group was slightly higher at baseline, with a larger values array, which can be a cause one of the reasons for an insignificant decrease in HOMA-IR values. NAFLD consists of two clinicopathological entities: a simple steatosis and NASH. Simple steatosis is detected as lipid accumulation in hepatocytes with little or no inflammation and fibrosis, while NASH comprises inflammation and fibrosis [46,47]. During the adipose tissue expansion, the modification of secreted adipokines towards a more steatogenic, inflammatory, and fibrogenic profile results with a higher production of cytokines. The excess of proinflammatory cytokines, and at the same time, a deficiency of anti-inflammatory cytokines has been observed in the progression of NASH in the liver and visceral adipose tissue [48]. With this trial, the biomarkers of inflammation were significantly reduced in both dietary groups. The AID group participants achieved greater reduction in TNF-α, and the CD group in IL-6. The reduction of hs-CRP was associated with the improvements in liver status in both dietary groups, and in the CD group, the reduction of IL-6 was associated with improvements of FLI. The significant reduction of inflammatory markers in both dietary groups can be explained by significant weight loss and by reduction of total and visceral adipose tissue, which is supported by the suggestion that the weight loss has a central role in reducing the inflammatory makers [49]. Additionally, it has been showed that, independent of the diet's composition, a hypocaloric diet had an anti-inflammatory effect [49], and by that, it may represent the most effective treatment for metabolic disorders by an effect on reducing the visceral adiposity, and the incidence of T2D, and the inflammation [49]. Both dietary groups in this study significantly and in similar quantities reduced their energy intake. However, the CD group slightly more, because they had higher energy intake at the baseline. Still, the reduction of energy intake by the AID group participants was significantly associated with improvements in FIB-4. A recent randomized controlled study examining two dietary strategies in subjects with obesity and NAFLD showed that the effect of weight loss in inflammatory markers might be greater when supplemented by a higher intake of fruits and vegetables [12]. The authors showed that the greater effect was achieved by a diet with high adherence to the Mediterranean diet [12]. In this trial, both dietary strategies had characteristics of the Mediterranean diet, thus overlapping in certain recommendations. Therefore, a higher intake of foods with anti-inflammatory characteristic was more promoted among the AID group participants, which is in detail described elsewhere [18]. Our study results are in line with the conclusion from a recent review and meta-analysis that a higher intake of fruits and vegetables leads to a reduction in proinflammatory mediators [50]. Fruits and vegetables abound with natural compounds that are found to be effective in the alleviation of NAFLD and its related comorbidities [51]. Specifically, these are flavonoids, which showed their protective effects in all stages of NAFLD prevention, development, complications, and consequences [51], although mostly observed in animal models, with experimentally induced hepatic steatosis and with higher doses that could be achieved with the usual diet. Each flavonoid, regardless of their diet sources, has its potential and biological effects, and a synergistic effect may be realised if these flavonoids are consumed together [51]. Furthermore, it was suggested that flavonoids may decrease body weight and fat deposition in visceral tissues and the liver, partly by increasing fatty acid β-oxidation and suppressing lipogenesis [51]. In this study, we showed total polyphenols and various flavonoids subgroups intake in both dietary groups, and their intake changes with the study trial.

After the period of 6 months of the intervention, the intake of total polyphenols and flavonoids increased in both dietary groups but significantly more in the CD group which could explain the improvement in the liver enzymes and NAFLD-FLS seen in this group. Although the AID group had significantly higher intake of flavonoids subgroups than the CD group, their intake was significantly associated with improvements in FLI and FIB-4 after the adjustments for potential confounders but not in NAFLD-FLS, which is seen in the CD group. The lack of significant associations with the intake of total polyphenols and flavonoids subgroups in the AID group could be explained by their relatively high intake at the baseline, compared with the CD group. There is still limited evidence on the association of polyphenols and specific flavonoids subgroups to NAFLD, NASH, and liver fibrosis, so this study results provide valuable information regarding this issue, particularly on the relationship between inflammatory markers and dietary strategies in the treatment of NAFLD.

There are some limitations and strengths in this study that should be recognized. This study included participants with obesity, with and without obesity-related complications, which includes NAFLD. NAFLD was evaluated using noninvasive techniques instead of a liver biopsy, which is currently the most reliable method for detecting steatohepatitis and fibrosis, specifically in subjects with NAFLD. In this study, adults with obesity participated, so the intent was to use noninvasive and rather fast parameters for NAFLD detection. Liver biopsy is a procedure that is limited by its cost and sampling errors and also with procedure-related complications [10], so we used scoring systems that need to be validated. However, we carried out a solid evaluation of liver status, and the design of this study was well protocoled regarding its procedure, methods used, specifically dietary methods that were clarified by the dietitian, and monitored for diet adherence, which resulted in the relatively low exclusion of the participants. Also, the concerns about monitoring adherence and sustainability of dietary intervention are overcome by frequent cooperation with the participants and by reviewing their 3-day food diary once per month in each dietary group according to dietary recommendations. The participants in both dietary groups had baseline average diet with slight anti-inflammatory potential, which all increased during the trial, the AID group participants significantly as expected. That increase of anti-inflammatory potential in the AID group was only significantly associated with improvements in liver fibrosis status, after the adjustments for confounders. Potential confounders that significantly reduced were obesity comorbidities such as metabolic syndrome components in the AID group. Furthermore, all participants in this trial were individuals with obesity, and although they all significantly reduced their baseline weight and adipose tissue, most of them remained in the obesity class after the 6 months of the trial. All of the above may be the reason that there were no observed significant associations of dietary change towards a more anti-inflammatory diet with improvements in liver status. Still, the observed alleviation of obesity comorbidities, including liver status, indicates their possible significant improvements if they continue with given anti-inflammatory dietary recommendations. Another important fact to point out is that our study participants were individuals with obesity younger than 50 years, meaning that among them, there were a specific number of individuals with so-called "metabolically healthy obesity," which is more often observed in young, physically active individuals, with better nutritional status and low levels of ectopic and visceral fat storage and not showing metabolic abnormalities, such as insulin resistance [52]. In addition to that, by detecting any liver parameters normality deviation and/or diagnosis of NAFLD in individuals with obesity at their younger age could prevent health complications in the future, along with reducing the costs of the medical treatments. To the present, it is still difficult to single out the effective diet or nutrient/s regarding NAFLD treatment; yet, the study results showed improvements in hepatic parameters associated with weight loss, reduction of total and visceral adipose tissue, and changes in energy and nutrients intake, specifically in flavonoid subgroup intake.

5. Conclusions

The study results showed the effectiveness of the anti-inflammatory diet in weight loss, in reducing the visceral adiposity and metabolic and inflammatory biomarkers and significant improvement of hepatic parameters in younger adults with obesity. Since there are still limited data about the specific dietary approach for ameliorating the NAFLD pathophysiology, the presented results may reinforce the effectiveness of nutrition-based lifestyle programs, with diet such as an anti-inflammatory dietary approach for the treatment and resolution of NAFLD.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

The data used to support the findings of this study are included within the article.

Conflicts of Interest

Dario Rahelić is the director of Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases at Merkur University Hospital, Zagreb, Croatia. He is the president of Croatian Society for Diabetes and Metabolic Disorders of Croatian Medical Association. He serves as an Executive Committee member of Croatian Endocrine Society, Croatian Society for Obesity and Croatian Society for Endocrine Oncology. He was a board member and secretary of IDF Europe and currently, he is the chair of IDF YLD Program. He has served as an Executive Committee member of Diabetes and Nutrition Study Group of EASD and currently, he serves as an Executive Committee member of Diabetes and Cardiovascular Disease Study Group of EASD. He has served as principal investigator or co-investigator in clinical trials of AstraZeneca, Eli Lilly, MSD, Novo Nordisk, Sanofi Aventis, Solvay, and Trophos. He has received honoraria for speaking or advisory board engagements and consulting fees from Abbott, Amgen, AstraZeneca, Bauerfeund, Bayer, Boehringer Ingelheim, Eli Lilly, Lifescan–Johnson and Johnson, Novartis, Novo Nordisk, MSD, Merck Sharp and Dohme, Mylan, Pfizer, Pliva, Roche, Salvus, Sanofi Aventis, and Takeda. Sanja Klobučar Majanović is the Vice President of Croatian Society for Diabetes and Metabolic Disorders of Croatian Medical Association and the Vice President of Croatian Society for Obesity of Croatian Medical Association. She serves as an Executive Committee member of Croatian Endocrine Society. She has served as principal investigator or co-investigator in clinical trials of Eli Lilly, MSD, and Sanofi Aventis. She has received honoraria for speaking or advisory board engagements and consulting fees from Abbott, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Lifescan–Johnson and Johnson, Novartis, Novo Nordisk, MSD, Merck, Sharp and

Dohme, Mylan, Pliva, and Sanofi Aventis. Other authors declare that they have no conflicts of interest.

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Review Article

Nonalcoholic Fatty Liver Disease and Cardiovascular Diseases: The Heart of the Matter

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Nonalcoholic fatty liver disease (NAFLD) has emerged as the most frequent cause of liver disease worldwide, comprising a plethora of conditions, ranging from steatosis to end-stage liver disease. Cardiovascular disease (CVD) has been associated with NAFLD and CVD-related events represent the main cause of death in patients with NAFLD, surpassing liver-related mortality. This association is not surprising as NAFLD has been considered a part of the metabolic syndrome and has been related to numerous CVD risk factors, namely, insulin resistance, abdominal obesity, dyslipidemia, hyperuricemia, chronic kidney disease, and type 2 diabetes. Moreover, both NAFLD and CVD present similar pathophysiological mechanisms, such as increased visceral adiposity, altered lipid metabolism, increased oxidative stress, and systemic inflammation that could explain their association. Whether NAFLD increases the risk for CVD or these diagnostic entities represent distinct manifestations of the metabolic syndrome has not yet been clarified. This review focuses on the relation between NAFLD and the spectrum of CVD, considering the pathophysiological mechanisms, risk factors, current evidence, and future directions.

1. Introduction

In the last decades, the prevalence of nonalcoholic liver disease (NAFLD) has been rapidly increasing [1]. NAFLD affects from 25 to 45% of the general adult population and up to 70% of type 2 diabetic patients in Europe and North America [2]. Nonalcoholic steatohepatitis (NASH) is a subtype of NAFLD characterized by progressive liver disease that can lead to liver cirrhosis and hepatocellular carcinoma. Most of the patients with NAFLD develop mild disease, although 20–30% of them progress to NASH [2]. Approximately 20% of the patients with NASH and progressive fibrosis will develop liver cirrhosis with an increased risk of hepatocellular carcinoma [3–5].

NAFLD is associated with multiple extrahepatic diseases ranging from mild to severe organ-specific-related complications. Patients with NAFLD usually associate features of metabolic syndrome (MetS) which overlaps with the cardiovascular risk factors. All these factors are involved in the development of cardiovascular events (CVEs), which are the most common causes of death among these patients [6]. Several prospective and retrospective studies confirmed the association between NAFLD and cardiovascular diseases (CVD), with a negative impact on patients' outcome [7, 8].

All these studies have investigated the association between NAFLD and CVD, and efforts have been made to establish a direct relationship between these two complex conditions. However, as both are multifactorial diseases sharing common risk factors, a direct causality relation between NAFLD and the development of CVD has not yet been firmly established [9]. Of note, a previous meta-analysis demonstrated that the risk of developing a CVEe is 64% higher in patients with vs. without NAFLD [10]. The method of NAFLD diagnosis (ultrasound or computed tomography) was the main limitation of the studies included in this meta-analysis. The lack of a histologically proven diagnosis of

NAFLD in the cross-sectional studies regarding cardiovascular involvement maintains the controversy on whether NAFLD is an active contributor or an innocent bystander in CVD development.

The complex physiopathology of both diseases with common risk factors and simultaneous involvement of different pathways makes it difficult to draw a clear conclusion regarding the direct relationship between NAFLD and CVD. Whether NAFLD confers any additional CVD risk, or whether an increase in CVD risk in NAFLD is due to associated CVD risk factors, is still uncertain. Confirming if NAFLD contributes as an independent CVD risk factor is important, as it is plausible that treatment of liver disease may decrease the CVD risk.

The aim of this review is to provide an update on the clinical evidence that supports the association between NAFLD and CVD, the impact on disease outcome, the main pathophysiological mechanism, and the most common cardiovascular comorbidities.

2. Pathophysiology of Cardiovascular Involvement in NAFLD

The pathophysiology behind the association of NAFLD with CVD is still incompletely understood and may involve other pathways besidesinsulin resistance (IR), such as oxidative stress, inflammation, and gut microbiota (Figure 1).

Abnormal glucose metabolism and hepatic IR are the major hallmarks of NAFLD, and they are the main elements in NAFLD and CVD pathogenesis [11–13]. The glucose metabolism disorders in NAFLD are secondary to the underlying systemic inflammation, visceral adiposity, and ectopic fatty tissue [14, 15]. The IR is associated with hyperinsulinemia that determines increase in hepatic glucose production and chronic hyperglycemia. Persistent hyperglycemia and IR promote oxidative stress, activation of inflammation, and dysregulation of lipoprotein metabolism [14, 16, 17]. IR promotes oxidative stress and activation of inflammatory signaling pathways, vascular inflammation, and dysregulation of lipids metabolism contributing to ectopic fat accumulation [14, 18, 19]. Pancreatic ectopic adipose tissue is also associated with IR and beta cell dysfunction, hyperinsulinemia, and secondary increase of free fatty acid level. Hyperinsulinemia and the decrease of hepatic insulin clearance secondary to NAFLD are associated with increased hepatic gluconeogenesis, hyperglycemia, and insulin overproduction, a pathological self-reinforcing cycle. Insulin, as a catabolic hormone, increases the production of various lipogenic enzymes by activating transcription factors as carbohydrate-responsive element binding protein (ChREBP) or sterol regulatory element-binding proteins 1c (SREBP-1c) [20]. The consequence is further accumulation of hepatic fat, overproduction of VLDL particles, and increasing the vascular atherogenetic process.

Atherogenic dyslipidemia associated with NAFLD is the consequence of increased de novo hepatic lipogenesis along with an elevated rate of lipid uptake, both mechanisms determining the overproduction and secretion of large triglyceride-enriched VLDL particles, including apolipoprotein C3 (ApoC3) and apolipoprotein B (ApoB). The atherogenic dyslipidemia is characterized by high serum triglycerides, low high-density lipoprotein (HDL) cholesterol, the predominance of small dense low-density lipoprotein (LDL) particles, and increased intermediate-density lipoprotein (IDL) [14, 21]. The atherogenic lipoproteins penetrate the vascular wall and activate the toll-like receptors (TLRs). These receptors sense the endogen damage signals and activate an immune response [22]. Activation of TLRs 2 and 4 receptors has a primary impact on activation of NOD-like receptor family, pyrin domain-containing protein 3 (NLRP3) inflammasome [14, 23]. NLRP3 inflammasome regulates the activity of enzyme caspase-1, known as interleukin (IL)-1β converting enzyme [24]. This complex mechanism leads to the activation of proinflammatory cytokines as IL-1β, IL-6, and C-reactive protein (CRP), all of them being involved in vascular inflammation and promoting atherosclerotic cardiovascular disease [24]. NAFLD patients have also an increased level of palmitic acid, incorporated in VLDL, and this saturated fatty acid also induces vascular inflammation by activating TLRs 2 and 4 [14, 25].

Endothelial dysfunction is one of the most important pathophysiological links between NAFLD and cardiovascular diseases. The oxidative stress and lipoprotein-mediated vascular inflammation are related to endothelial dysfunction that is characterized by decreased bioavailability of the nitric oxide (NO) [14, 23, 26, 27]. There are two main factors contributing to endothelial dysfunction in NAFLD patients: low NO availability and hyperhomocysteinemia. It was demonstrated that patients with NAFLD have a low level of asymmetric dimethyl arginine (ADMA) determined by decreased liver breakdown of this molecule. ADMA is an endogenous antagonist of nitric oxide synthase (NOs) and its elevation is associated with decreased NO. Hyperhomocysteinemia induces oxidative stress by reduced glutathione stores in direct relation with low levels of NO. All these endothelial abnormalities increase platelet activation and vascular resistance [28–30].

In patients with NAFLD, an *imbalanced coagulation* cascade was demonstrated, and these subjects are being prone to a hypercoagulable state due to high levels of coagulation factors FVIII, FIX, FXI, FXII, fibrinogen, von Willebrand factor, and plasminogen activator inhibitor-1, along with low levels of anticoagulant factors as antithrombin III and protein C [12, 26, 30].

Recently, the hepatokines have been demonstrated to be potential mediators of cardiometabolic syndrome in NAFLD [31]. Of these, fetuin A was associated with CVD [32]. The experimental studies have demonstrated that fetuin A induces low-grade inflammation in concert with fatty acids [33].

Fat accumulation in the liver could be associated with ectopic fatty tissue, including myocardial fat and adipose tissue surrounding the heart, a central aspect of the relationship between NAFLD and CVD [34]. Under physiological conditions, this adipose tissue has anti-inflammatory and antifibrotic proprieties [35, 36]. In NAFLD, the systemic

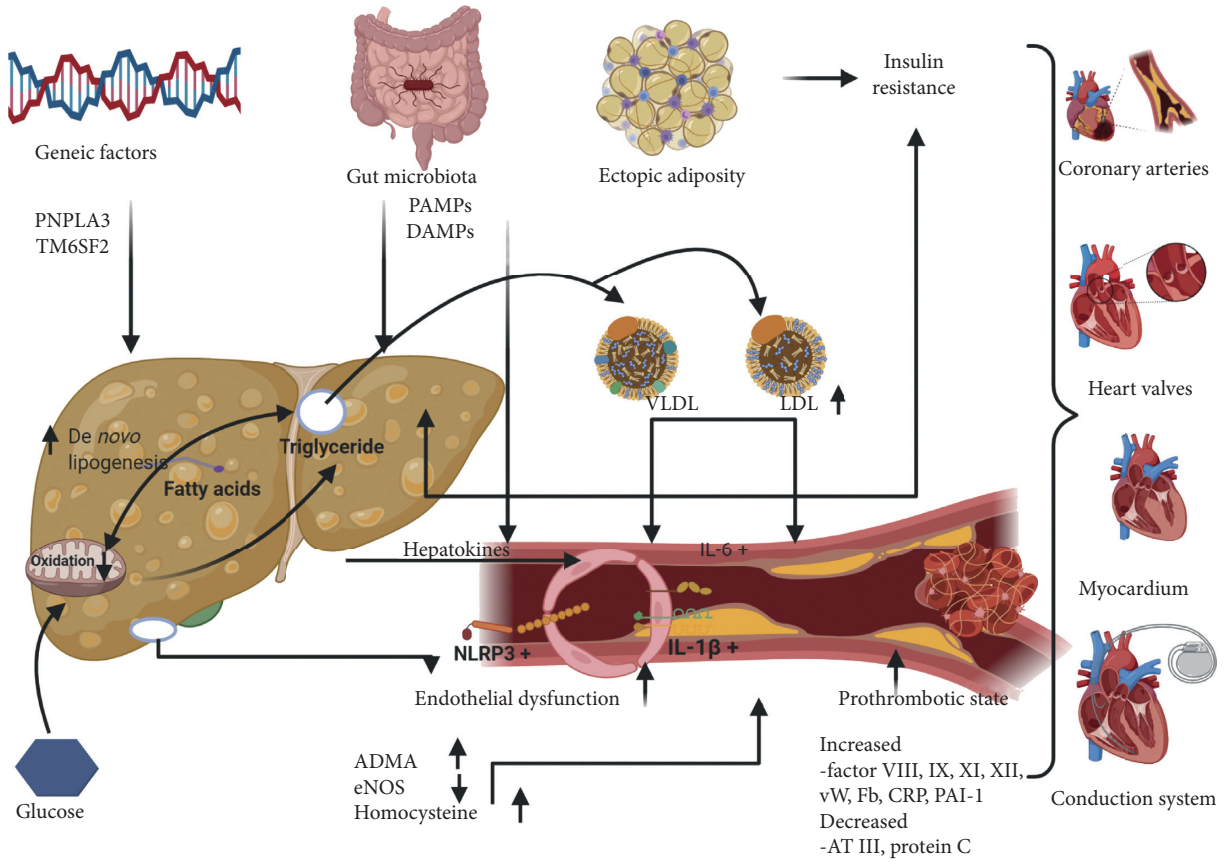

Figure 1: Pathophysiology of cardiovascular involvement in NAFLD.

inflammatory syndrome is changing the epicardial adipose tissue phenotype, transforming these cells in activated adipose cells that secret proinflammatory cytokines, activate profibrotic pathways, and promote ventricular fibrosis and inflammation [35–37].

The previous studies demonstrated that NAFLD has also a *genetic predisposition*. The polymorphism in the patatin-like phospholipase domain-containing 3 (PNPLA3) and the transmembrane 6 superfamily member 2 (TM6SF2) genes are associated with NAFLD, NASH, fibrosis, and an increased risk of hepatocellular carcinoma [38]. PNPLA3 I148 M and TM6SF2 E167 K are variants that interfere with hepatic triglyceride metabolism [39, 40]. Both variants are predisposing the patients to NAFLD and are associated with increased disease severity [41]. Interestingly, carriers of genetic variants of PNPLA3 and TM6SF2 tend to have cardioprotective phenotype [42, 43], although the precise in vivo physiological role of PNPLA3 remains incompletely characterized.

Recently, *gut microbiota* was demonstrated as a contributing factor for atherosclerosis, T2DM, and NAFLD [44, 45]. The impaired gut mucosal barrier permits pathogen-associated molecular patterns (PAMPs) and damaged-associated molecular patterns (DAMPs) entering the systemic circulation, inducing a gut-related inflammatory response [46, 47]. NAFLD and advanced fibrosis are associated with an increased concentration of *Escherichia coli* bacteria, *Ruminococcus*, and *Blautia* and a decrease in *Firmicutes* strains [48–50]. This profound intestinal dysbiosis is independent of IR and obesity and is related to increased gut-derived metabolites as 3-(4-hydroxyphenyl)-lactate or phenylacetate. Also, gut-derived microbiota and its metabolites were recently demonstrated as potentially important players in cardiovascular disease pathophysiology [51].

All this evidence supports the important role of the liver in the pathophysiologic processes of CVD development, although an independent link between NAFLD and coronary arterial disease and atherosclerosis remains difficult to confirm.

3. Cardiovascular Risk Assessment in NAFLD

NAFLD shares many risk factors with CVD, most notably insulin resistance, obesity, and dyslipidemia. Also, NAFLD itself likely influences CVD development, by means of hypertriglyceridemia and induction of a hypercoagulable state [10, 30, 52].

Several studies demonstrated that all stages of NAFLD are associated with increased CVEs as acute coronary syndrome, stroke, or malignant arrhythmias. Moreover, compared with patients without NAFLD, those with fatty liver have an elevated risk of CVEs independent of the presence of MetS or T2DM [6, 53]. Even in normoponderal patients, ultrasound-defined NAFLD is correlated with a high incidence of CVEs, concluding that NAFLD acts independently of overweight and obesity [54].

Recent data also suggested that patients with NAFLD had a twofold increase in the risk of developing CVEs, and in those with liver fibrosis this risk was doubled [6].

As a systemic progressive disease, NAFLD increases the risk of CVD, although the most commonly used cardiovascular risk factor scoring system for cardiovascular risk management, such as the Framingham Risk Score or SCORE, may underestimate cardiovascular risk in this special patient category. No validated CVD risk score specific for NAFLD patients has yet been validated.

The most important clinical practice issue is that NAFLD diagnosis could be associated with an additional risk for CVD when concomitant atherosclerotic risk factors are already diagnosed, although before including NAFLD in new cardiovascular risk scores we should establish a consensus on how to quantify and qualify NAFLD severity. Until then, the use of classical risk factors is adequate to evaluate CVD risk in NAFLD patients, as Treeprasertsuk et al.demonstrated. In this study, the Framingham Risk Score had a good sensibility in identifying coronary heart disease risk in a cohort with more than 300 NAFLD patients followed by a mean of 11.5 years [55].

An important clinical question is if NAFLD indicates the need for an extensive cardiovascular risk assessment independently of the presence of classical risk factors. Many cross-sectional studies confirmed the independent associations between NAFLD and the presence of subclinical vascular disease or changes in heart morphology such as left ventricular hypertrophy or diastolic dysfunction. However, there is no prospective evaluation showing an additional role of these imaging tests in CVD risk evaluation. Therefore, there is not enough evidence to routinely recommend imaging tests for subclinical vascular or heart disease based on the presence of NAFLD. The association between NAFLD and CVD is further described in Table 1.

3.1. Impact of NAFLD on Cardiovascular Disease Outcome. Several meta-analyses reported conflicting results regarding cardiovascular mortality rate in patients with NAFLD. A meta-analysis including 16 studies demonstrated an increased risk of CVEs in NAFLD patients compared to those without NAFLD [10]. However, the CVD-related mortality was higher only in patients with NASH and high fibrosis scores or high histological fibrosis stage. A second meta-analysis found an increased liver-related mortality in patients with NAFLD, with no correlation with CVD-related mortality [72]. Moreover, a meta-analysis of 34 studies including more than 160,000 patients by Wu et al. was unable to confirm a correlation between the presence of NAFLD and increased cardiovascular mortality [73]. The major limitation of these meta-analyses lies in the heterogeneity of diagnosis criteria of NASH. However, in consideration of all studies, the meta-analysis of Wu et al. confirmed that NAFLD was associated with an increased risk for incident CVD (HR 1.37; 95% CI 1.10–1.72) and that NAFLD patients were more likely to develop coronary heart disease (HR 2.31; 95% CI 1.46–3.65) and hypertension (HR 1.16; 95% CI 1.06–1.27) [73]. In addition, it was demonstrated that the severity of NAFLD was a major determinant of increased risk of CVD [74]. A comprehensive meta-analysis performed by Younossi et al.that included 86 studies, with a sample size of 8,515,431 patients, reported a pooled CVD-related mortality rate in patients with NAFLD of 4.8 per 1000 person-years [75].

Current evidence shows that NAFLD is associated with an increased risk for CVD and CVEs. Patients with NASH and advanced fibrosis as well as NAFLD patients with concomitant T2DM can be identified as being part of a special risk group [76–78].

Ekstedt et al., in a study with a mean follow-up duration of 26.4 years, stated that patients with NAFLD presented higher mortality than patients from the general population (HR: 1.29; 95% CI 1.04–1.59) [43]. The authors identified CVD as well as liver-related disease to be the main causes of death in patients with NAFLD. Patients with more advanced fibrosis stage presented increased mortality (HR 3.3, CI 2.27–4.76, $P < 0.001$) [8]. A prospective study including 898 patients that were screened for steatosis by ultrasound found that CVEs, defined as ischemic stroke, myocardial infarction, revascularization procedures, newly diagnosed arterial fibrillation, and cardiovascular death, were associated with NAFLD. The authors also concluded that the presence of NAFLD determined a 2-fold increase in the risk of CVEs. The patients with liver fibrosis presented a higher, 4x increase in the risk for development of CVEs. Kim et al., in a large study comprising 11,154 patients among whom 34% were diagnosed with NAFLD, reported that fibrosis but not NAFLD was associated with increased mortality. CVD represented the main cause of death [79]. A more recent meta-analysis that included 108,711 patients with NAFLD, 44% women and 56% men, showed that CVEs and mortality were twice higher in women than in men (OR 2.12, 95% CI 1.65–2.73, $P < 0.001$) [80].

While simple steatosis alone confers less cardiovascular risk than NASH, the individual overall cardiovascular risk results from the combination of NAFLD stage and cardiometabolic risk factors.

4. Cardiovascular Comorbidities in NAFLD

4.1. NAFLD and Atherosclerosis. Atherosclerosis is defined by the development of neointimal cholesterotic plaques in large arteries and is directly associated with acute coronary syndrome and stroke.

Several studies have demonstrated that NAFLD is a risk factor for atherosclerosis and, therefore, associated with increased prevalence of ischemic heart disease [81–85]. Atherosclerosis has been extensively documented in patients with NAFLD and subclinical markers of atherosclerosis such as coronary artery calcium (CAC) score [86, 87], as well as carotid intima-media thickness (cIMT) [88–90] or arterial stiffness via brachial-ankle index, have been used to confirm this association. Prospective studies have demonstrated that NAFLD patients are associated with higher CAC scores than those without NAFLD [91, 92], even among patients with normal body mass index (BMI). The annual rate of CAC progression and the cIMT were higher in NAFLD patients

Table 1: Current evidence of the association between NAFLD and CVD.

Authors, year	Country	Type of study	Main characteristics	NAFLD diagnostic	Results
Bonnet et al., 2017 [56]	France	Prospective, cohort	2,565 patients, normotensive, followed up for 9 years	GGT, FLI	GGT was associated with incident hypertension (standardized odds ratio: 1.21; 95% confidence interval (1.10–1.34); $P = 0.0001$). FLI predicted incident hypertension in a multivariable model.
Huh et al., 2015 [57]	South Korea	Prospective, cohort	1,521 patients, aged 40–70, followed up for 2.6 years	FLI	10.06% of patients developed hypertension; FLI was associated with baseline blood pressure and was an independent risk factor for hypertension.
Lau et al., 2010 [58]	Germany	Prospective, cohort	3191 patients, aged 20–79, followed up for 11.6 years	US and liver Enzymes	Fatty liver disease was associated with hypertension at baseline and at follow-up, OR 2.8; 95% CI 1.3–6.2 and OR 3.1; 95% CI 1.7–5.8, respectively.
Ryoo et al., 2014 [59]	South Korea	Prospective, cohort	11350 patients, only men, aged 30–59, normotensive, followed up for 5 years	US	58.2% of the participants developed prehypertension, 63.7% of the patients with mild NAFLD, and 70.3% of the ones with severe NAFLD, $P < 0.001$.
Sung et al., 2014, [60]	South Korea	Retrospective, cohort	11448 patients, aged 42.1 ± 6.8, normotensive, followed up for 5 years	US	NAFLD was associated with incident hypertension, after adjustment for multiple confounders [aOR = 1.60 (95% CI 1.30, 1.96; $P < 0.001$)].
Agac et al., 2013 [61]	Turkey	Prospective, cross-sectional	80 patients with acute coronary syndrome	US	NAFLD was present in 81.2% of the patients with acute coronary syndrome; multivariate analysis found NAFLD to be associated with higher SYNTAX score (OR, 13.20; 95% CI, 2.52–69.15).
Agarwal et al., 2011 [62]	India	Prospective, cross-sectional	124 patients with T2DM	US	CAD was diagnosed in 60.5% of the patients with NAFLD and in 45.2% of the ones without NAFLD.
Arslan et al., 2012 [63]	Turkey	Prospective, cross-sectional	151 patients with newly diagnosed CAD, without T2DM	US	NAFLD was diagnosed in 64.9% of the patients. Presence of NAFLD was associated with poor coronary collateral development.
Chan et al., 2014 [64]	Malaysia	Prospective, cross-sectional	399 diabetic patients, mean age 62.8 ± 10.5	US	NAFLD was found in 49.6% of patients but was not associated with IHD.
Chen et al., 2010 [65]	Taiwan, China	Retrospective, cross-sectional	295 patients	US, CT	NAFLD (OR, 2.462; 95% CI, 1.065–5.691) was found to be an independent factor for the risk of coronary artery calcifications.
Chiang et al., 2010 [66]	Taiwan, China	Retrospective, cross-sectional	724 patients	US	NAFLD was found to be an independent predictor for future CVD risk ≥10% (OR: 1.89, $P = 0.004$).
Keskin et al., 2017 [67]	Turkey	Retrospective, cohort	360 patients with STEMI	US	Multivariate analysis found grade 3 NAFLD to be a risk factor for in-hospital mortality (OR 4.2).
Perera et al., 2016 [68]	Sri Lanka	Prospective	120 patients with acute coronary syndrome	US	NAFLD was identified in 46.7% of the participants. NAFLD was associated with a higher predicted in-hospital mortality (adjusted OR 31.3, CI 2.2–439.8, $P = 0.011$) and at 6 months after discharge (adjusted OR 15.59, CI 1.6–130.6, $P = 0.011$).

Table 1: Continued.

Authors, year	Country	Type of study	Main characteristics	NAFLD diagnostic	Results
Wu et al., 2017 [69]	China	Cross-sectional	2345 patients	US	NAFLD was significantly associated with the development of coronary artery calcifications (adjusted OR: 1.348, 95% CI: 1.030–1.765).
Baratta et al., 2020 [6]	Italy	Prospective	898 patients, followed up for 41.4 months	US	Patients with NAFLD presented over 2x increase in risk of CVEs; patients with liver fibrosis had a 4x increase in risk.
Pastori et al., 2020 [70]	Multicenter	Prospective, cohort	1735 patients with nonvalvular atrial fibrillation	FLI	NAFLD was diagnosed in 42.2% of the participants but was not associated with bleeding or with thrombotic risk.
Alexander et al., 2019 [71]	Multicenter (Italy, Netherlands, Spain, United Kingdom)	Matched cohort study	120795 patients with NAFLD or NASH	/	NAFLD was not found to be associated with increased risk for acute myocardial infarction.

NAFLD: nonalcoholic fatty liver disease; FLI: fatty liver index; US: ultrasound; OR: odds ratio; CI: confidence interval; HR: hazard ratio; CAD: coronary artery disease; T2DM: type 2 diabetes mellitus; CAD: coronary artery disease; IHD: ischemic heart disease; CT: computed tomography; CVD: cardiovascular disease; STEMI: ST segment elevation myocardial infarction; CVE: cardiovascular event; NASH: nonalcoholic steatohepatitis.

independent of obesity, dyslipidemia, or T2DM [86, 93, 94]. Also, increased cIMT was associated with the presence of liver fibrosis assessed by fibrosis-4 (FIB4) and aspartate transaminase to platelet ratio index (APRI) scores [95]. NAFLD is associated with plaques development not only in coronary arteries but also in carotid arteries, iliac arteries, or celiac trunk [96], with predisposition to multiarterial calcifications.

Moreover, NAFLD has also been associated with endothelial dysfunction [97] as well as with unstable coronary plaques [98] explaining the high risk of ischemic events in these patients [3]. Furthermore, patients with ST segment elevation myocardial infarction (STEMI) presented higher short-term mortality and worse long-term prognostic when NAFLD was associated [67].

A meta-analysis including more than 85,000 patients demonstrated that subclinical atherosclerosis was significantly more frequent in those patients diagnosed with NAFLD (OR = 1.60, 95% CI: 1.45–1.78) [89].

NAFLD increases the atherosclerotic risk and makes the patients prone to the development of unstable plaques adding to cardiovascular risk factors as dyslipidemia, obesity, arterial hypertension, and T2DM.

4.2. NAFLD and the Cardiac Structure. NAFLD has been associated with structural heart disease. Diastolic dysfunction and heart failure with a preserved ejection fraction and increased myocardial remodeling are common findings in patients with NAFLD [35]. These changes, together with an increased risk of aortic sclerosis [99], can lead to the development of arrhythmias and the increased risk for CVD events [100].

4.3. NAFLD and Arrhythmias. NAFLD has been associated with increased risk of atrial fibrillation [101] and prolonged QTc interval [102]. The physiopathological mechanisms that lead to arrhythmias in patients with NAFLD include the increase of the epicardial adiposity which associates a rise in proinflammatory adipocytokines, followed by the development of myocardial fibrosis [35]. Targher et al., in a prospective study including diabetic patients, reported a high risk for atrial fibrillation when NAFLD was associated, with an OR of 4.49 for a 95% CI between 1.6 and 12.9 [101]. Another prospective study comprising patients followed up for 16.3 years reported an independent association between NAFLD and atrial fibrillation, with an adjusted OR of 1.88 for a 95% CI between 1.03 and 3.45 [103]. Ventricular arrhythmias were also associated with NAFLD, in a retrospective study on patients with type 2 diabetes that underwent 24-hour Holter monitoring. After adjusting for confounders, the authors reported an OR of 3.01 for a 95% CI between 1.26 and 7.17 [104].

4.4. NAFLD and Hypertension. The relation between NAFLD and hypertension has not yet been fully explained. There are indications that the systemic inflammation associated with NAFLD could promote the activation of the sympathetic nervous system and, thus, induce hypertension [105]. Moreover, insulin resistance would promote hypertension via the augmentation of free fatty acids that lead to perivascular fat deposits situated in the vicinity of vessels and the renal sinus. Furthermore, the high levels of homocysteine found in the setting of NAFLD can, together with gut dysbiosis, induce the increase in oxidative stress and thus promote hypertension [106]. Although several studies demonstrated an association between NAFLD and hypertension [57–59, 107], there was considerable heterogeneity concerning the criteria used for the diagnosis of NAFLD.

While some studies used ultrasonography for the diagnosis of NAFLD [58, 59], others used magnetic resonance imaging (MRI) [108] or surrogate scores such as fatty liver index (FLI) [57]. Lau et al., in a prospective study including 3191 patients from Germany, concluded that the subjects diagnosed with NAFLD presented a higher risk of hypertension than patients without NAFLD, reporting an OR of

3.1 for a 95% CI of 1.7–5.8 [58]. Another larger prospective study from South Korea, including 11,350 male patients, found a higher risk for prehypertension in patients with NAFLD. Interestingly, the risk varied according to NAFLD severity [59]. Huh et al. evaluated the risk for hypertension in a prospective longitudinal study including 1,521 patients without CVD. The authors found that the risk for hypertension was higher in the NAFLD group as diagnosed by FLI and that the risk increased gradually in accordance with the FLI value [57]. More recently, Lorbeer et al., in a study that used MRI in order to measure the hepatic fat fraction, reported an association of the liver fat content with high blood pressure as well as with higher odds of hypertension [107].

4.5. CVD Events and Associated Mortality in Patients with NAFLD. Numerous studies and meta-analysis have found CVD events to be associated with NAFLD and CVD-related death has been considered the main cause of mortality in these patients [8, 108]. The risk of death following a CVD event in patients with NAFLD was also analyzed in a recent meta-analysis including 16 studies with a total of 34,043 patients. The pooled results indicated an increased risk for fatal and nonfatal CVD events in patients with NAFLD, with an OR of 1.64, 95% CI 1.26–2.13, but the direct causality between NAFLD and CVD events could not be demonstrated because of the observational design of the studies included [100]. A recent study performed by Paik et al. using mortality data from the National Vital Statistics System multiple-cause mortality data between 2007 and 2016 identified 353,234 patients diagnosed with NAFLD. The authors concluded that CVD was the second most frequent cause of death in these patients, following liver cirrhosis [109].

A recent large retrospective study carried out in Germany and involving 111,492 patients showed an increased risk of myocardial infarction when NAFLD was associated, with a hazard ratio of 2.14 (95% CI 1.59, 2.89) [110]. A comprehensive analysis of 285 patients with biopsy-proven NAFLD monitored for 5.2 years showed that advanced fibrosis was a predictor of CVD events and that the NAFLD fibrosis score was the only noninvasive predictor of CVD [111]. However, there is still some controversy regarding the risk of ischemic events in the setting of NAFLD. A matched cohort study of 18 million Europeans including patients from electronic primary healthcare databases from Italy, Netherlands, Spain, and the United Kingdom did not find an increased risk of myocardial infarction or of stroke in patients with NAFLD [71]. However, the design of the study presented the risk for misclassification of the disease; thus, the results should be interpreted with caution.

5. Unmet Needs

Long-term assessment of a larger number of histologically diagnosed patients is needed to understand the causes of mortality in NASH and the direct relation with CVD-related events and mortality. Further studies should focus on the role of NAFLD-associated inflammation as a new cardiovascular risk. Also, future studies should be aimed at unraveling the role of other NAFLD-mediated pathways, such as hepatic inflammation, in the pathogenesis of atherosclerosis.

In addition, it is important to better clarify how NAFLD progression from steatosis to more severe disease influences the metabolic and inflammatory components that may associate this disease with atherosclerosis. Prospective long-term studies with homogeneous diagnostic criteria, considering not only the presence but also the severity of NALFD, are necessary to test if this diagnosis can improve cardiovascular disease risk stratification. The greatest challenge would be to separate it from its aggravating metabolic consequences that characterize the MetS, like atherogenic dyslipidemia.

In the meantime, considering the associated higher cardiovascular risk, weight loss, exercise, and control of concomitant established risk factors for atherosclerosis are mandatory in individuals with NAFLD.

6. Conclusions

The causal relationship between CVD and NAFLD remains under investigation, but the strong bidirectional association between CVD and NAFLD warrants clinical intervention in patients with NAFLD to modify metabolic risk factors, including T2DM, dyslipidemia, hypertension, and obesity.

Although current cardiovascular society guidelines have not identified NAFLD as an independent risk factor for CVD despite recent studies suggesting NAFLD's role in incident CVD, vigilant age-appropriate screening and treatment for associated risk factors, including weight loss for obesity, glycemic control for T2DM, and treatment of hypertension and hyperlipidemia, remain prudent strategies that should be supported by clinicians managing patients with NAFLD.

Cardiologists should be aware that patients with CVD may have progressive forms of NAFLD, while hepatologists should be aware that patients with progressive NAFLD have a markedly increased risk of CVD. All physicians should perform correct cardiovascular risk management, in a multidisciplinary setting, all these in the best interest of the patients.

Abbreviations

ADMA: Asymmetric dimethyl arginine
ATIII: Antithrombin III
CRP: C-reactive protein
DAMPs: Damaged-associated molecular patterns
Fb: Fibrinogen
IL-6: Interleukin 6
IL-1β: Interleukin 1β
LDL: Low-density lipoprotein
NLRP3: Pyrin domain-containing protein 3
PAI-1: Plasminogen activator inhibitor-1
PAMPs: Pathogen-associated molecular patterns
PNPLA3: Patatin-like phospholipase domain-containing 3
TM6SF2: Transmembrane 6 superfamily member 2
VLDL: Very low-density lipoprotein
vW: Von Willebrand factor.

Data Availability

The data used to support this study are included within this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors contributed equally to this review. All authors have read and agreed on the published version of the manuscript.

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Review Article

Association between Nonalcoholic Fatty Liver Disease and Endocrinopathies: Clinical Implications

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Nonalcoholic fatty liver disease (NAFLD) has a rising prevalence worldwide. Its potential for evolution towards liver cirrhosis and hepatocellular carcinoma, as well as associations with extrahepatic manifestations, represents a double burden for patients and physicians alike. Recently, there has been increasing evidence of the association between NAFLD and a number of endocrinopathies, such as hypothyroidism, polycystic ovarian syndrome (PCOS), hypopituitarism, growth hormone deficiency (GHD), hypogonadism, and hypercortisolism. Definite correlations are supported by clear evidence so far, but further studies are needed in order to completely clarify the pathogenic mechanisms and, especially, to identify therapeutic implications. In this review, we present the main relationships between NAFLD and endocrinopathies, emphasizing the reciprocal causality, evolutive interconnections, and current clinical scenarios of presentations of which the clinicians should be aware.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most important nonneoplastic pathologies in contemporary medicine, being the most common cause of chronic liver disease worldwide. It is characterized by the accumulation of fat in the liver, histologically being identical to alcoholic liver disease, in patients without significant alcohol consumption [1].

The clinical importance of NAFLD is related to its prevalence of up to 30% in the general population, thus exceeding that of viral hepatitis and alcoholic liver disease [2, 3]. It is worrying that the disease includes not only a form considered benign (hepatic steatosis) but also progressive forms (steatohepatitis with or without fibrosis) possibly evolving towards liver cirrhosis and, in some cases, hepatocellular carcinoma [4]. Moreover, the pathogenic mechanisms of progression from the simple form to the aggressive ones are not completely elucidated due to their complexity, through the involvement of multiple processes as well as metabolic, immunological, and genetic imbalances [5]. NAFLD is a topical issue in terms of diagnosis and treatment for a variety of specialties. One element that certifies the importance of nonalcoholic fatty liver is the fact that, in the coming years, this pathology is expected to become the first indication for liver transplantation, surpassing viral liver cirrhosis of any etiology [6].

In recent years, there has been increasing evidence of the association between NAFLD and a number of endocrinopathies, such as hypothyroidism, polycystic ovarian syndrome (PCOS), hypopituitarism, growth hormone deficiency (GHD), hypogonadism, and hypercortisolism [7]. The relationship between NAFLD and these hormonal abnormalities is not yet completely understood as their role in the pathogenesis of NAFLD has not been established so far.

However, despite the fact that NAFLD is an increasingly common disease, it is often overlooked by endocrinologists

and is assessed only by hepatologists. Given NAFLD's long-term clinical impact, it is important for practicing physicians, endocrinologists, and hepatologists to detect forms of NAFLD associated with endocrine diseases.

In this review, we tried to include the most important up-to-date information on the association of nonalcoholic fatty liver disease and various endocrinopathies, with specific reference to their epidemiology, pathophysiological mechanisms, and treatment principles.

2. NAFLD and Hypothyroidism

The thyroid gland plays a key role in the regulation of various metabolic processes and its dysfunction is linked to diverse diseases. Disorders in thyroid hormones concentration may lead to insulin resistance, obesity, and hyperlipidemia, which are well-known risk factors for the development of NAFLD [8]. Thyroid-stimulating hormone (TSH) can directly increase hepatic gluconeogenesis and decrease 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase phosphorylation inducing hypercholesterolemia [9]. Moreover, oxidative stress is a common pathway for both hypothyroidism and NAFLD [10]. In 2014, in a systematic review, the authors found a prevalence of hypothyroidism ranging between 15.2% and 36.3% among patients with NAFLD [11], while the national estimated prevalence of hypothyroidism in the United States population was 3.7% [12]. In one particular study which analysed patients with biopsy-proven NAFLD, the prevalence of hypothyroidism was higher compared to matched controls (21% vs. 9.5%, resp.), regardless of age, obesity, hyperlipidemia, and diabetes [13]. This association seems to be more than a mere coincidence, since hypothyroidism may be involved in the development of NAFLD. Thus, a recent systematic review and meta-analysis including over 42000 patients from 13 studies found a high correlation between hypothyroidism and NAFLD, providing strong epidemiological evidence regarding the risk for NAFLD for both subclinical and overt hypothyroidism, compared to euthyroid subjects [14]. Overt hypothyroidism, defined as increased TSH and low free T4 (FT4), was more significantly correlated with NAFLD than subclinical hypothyroidism, stated as increased TSH and normal FT4, probably due to the combined concomitant effects of low thyroid hormones and higher TSH level [14]. Subclinical hypothyroidism was connected to NAFLD in a dose-dependent manner; even in the range of normal TSH levels, association with NAFLD was reported independently of other recognized metabolic risk factors [15]. Moreover, "low-normal" thyroid function was reported as a risk factor for advanced fibrosis [16]. Recently, the role of thyroid hormone receptor in hepatic stellate cells activation was postulated [17], but it still remains unclear if or how exactly thyroid dysfunction is supposed to accelerate the progression of NAFLD to steatohepatitis and, consequently, to advanced fibrosis. Thyroid hormone replacement therapy leads to a significant decrease of serum lipids and has a favourable effect on overweight or obesity [18]. Levothyroxine administered for 15 months in patients with subclinical hypothyroidism showed benefit on serum transaminases and ultrasound-diagnosed NAFLD [19]. Decrease of hepatic fat content measured by magnetic resonance spectroscopy was demonstrated after low-dose levothyroxine administered for 4 months in patients with normal thyroid function, type 2 diabetes, and NAFLD [20]. However, it is not yet known if thyroid replacement therapy in patients with NAFLD will improve the current status of the disease or stop its progression, as more studies are needed to elucidate the interrelation between NAFLD and hypothyroidism. Nevertheless, until further evidence, NAFLD patients' surveillance should be carried out by annual TSH testing.

3. NAFLD and Polycystic Ovarian Syndrome

Polycystic ovarian syndrome (PCOS) is a reproductive disease characterized by hyperandrogenism with polycystic ovarian and oligomenorrhea, after exclusion of other endocrine disorders [1]. PCOS is the most common cause of anovulatory infertility, affecting 5% to 18% of the reproductive aged women worldwide, while it may also lead to additional health problems in adulthood, with long-term consequences [21]. This disease is associated with risk factors for cardiovascular disease, obesity (60%), hepatic steatosis (50%), and insulin resistance (IR) (70%) [22].

An increasing number of cohort studies strongly suggest that the prevalence of NAFLD is remarkably high in young women with PCOS, regardless of the presence of obesity or other features of the metabolic syndrome [4]. Brown et al. described NAFLD for the first time after a liver biopsy for a 24-year-old woman with PCOS, obese, without a history of alcohol consumption, diabetes, or other known liver diseases which are consistently associated with increased transaminases [23].

Lim and Bernstein showed that the prevalence of NAFLD in PCOS varies from 35 to 70%, compared with 20 to 30% in women that do not present with PCOS, of similar age, BMI, and hip circumference [24]. Ramezani-Binabaj et al. reported in a meta-analysis that patients with PCOS had a 3.93-fold increased risk of coexisting NAFLD independent of BMI [25]. In particular, some case-control studies have also reported that PCOS is very common among young women with biopsy-proven NAFLD. In these patients, the prevalence of PCOS ranged from about 50% to 70%, at the same time being more likely to have the most severe histological forms of NAFLD [26]. A recent meta-analysis of 17 observational studies, which included 2734 women with PCOS and 2561 healthy women of similar age and BMI, showed that young women with PCOS had twice the risk of prevalent NAFLD than control women [27].

According to the Rotterdam diagnostic criteria for the three PCOS phenotypes (classical, ovulatory, and normal androgen), studies have shown that the risk of insulin resistance and metabolic syndrome is the highest in women with PCOS and classical phenotype, intermediate in those with ovulatory phenotype, and the lowest in those with normo- and rogenic phenotype [28, 29].

Over time, there has been an ongoing debate about mechanisms underlying the alleged involvement of PCOS in

NAFLD development and progression. There is ample scientific evidence to suggest that PCOS and NAFLD have common multifactorial pathophysiological mechanisms, representing a complex interaction between abdominal adiposity/overweight/obesity, systemic insulin resistance, chronic inflammation, and hyperandrogenism [4]. In 1980, Burghen et al. showed that patients with obese PCOS associate hyperinsulinemia, a hypothesis later confirmed in other studies, which have even proved that it is independent of obesity [30, 31]. The association of PCOS with increased tolerance to glucose and diabetes, abdominal adiposity, and dyslipidaemia, as well as with metabolic syndrome, led to its classification as a metabolic disorder [32].

The main presumed reasons why PCOS women may have low insulin sensitivity include both defects in the insulin receptors found on the surface of the ovaries and irregular insulin signalling which increases androgen production in theca cells, the primary source of excessive androgen biosynthesis in women with PCOS [33]. Thus, IR leads to compensatory hyperinsulinemia, which stimulates theca cells in LH-sensitized ovaries to secrete testosterone and androstenedione. Baranova et al. showed in a systematic analysis that IR is present in 50%–80% of women with PCOS and NAFLD [34]. In addition, multiple studies have shown that PCOS women with hepatic steatosis have high levels of IR compared to PCOS women without steatosis [1, 3].

Another theory that explains the pathogenic mechanism between NAFLD and women with PCOS refers to the effects of hyperandrogenism on low-density lipoprotein (LDLR) receptors, in which androgens suppress LDLR gene transcription to prolong the half-life of very low-density lipoproteins (VLDL) and LDL, thus causing the accumulation of lipids in the liver [35]. Kumarendran et al. demonstrated in a retrospective cohort study, which included over 63000 women with PCOS and 121000 age-, BMI-, and location-matched women, that PCOS subjects had an increased incidence of NAFLD, hyperandrogenism being a risk factor for NAFLD development in women with PCOS [36].

Currently, more studies are needed to demonstrate the best management of NAFLD in women with PCOS. The first recommended therapeutic option for NAFLD in women with PCOS is to change their lifestyle based on a low-calorie diet and increased physical activity. The second option is the association between a lifestyle change and metformin [29, 37]. Evidence has recently emerged of the benefits of pioglitazone, liraglutide, and other glucagon-1 peptide-1 receptor agonists for decreasing intrahepatic fat content in the treatment of women with PCOS and NAFLD [38, 39].

Given the increasing prevalence of NAFLD in young women with PCOS and the high risk of developing long-term liver complications, systematic screening of NAFLD in patients with PCOS should be considered, especially whenever hyperandrogenism and metabolic syndrome are present. Unfortunately, the optimal method of screening in this population is currently unknown, but monitoring of aminotransferase and hepatic steatosis levels by ultrasound, especially in those with metabolic syndrome, can be a start.

4. NAFLD and Hypopituitarism

Hypopituitarism is a chronic endocrine disorder defined as the lack or diminution of pituitary gland function, due to pituitary or hypothalamic pathologies. There are two types of hypopituitarism, classified by the main causal factor: primary hypopituitarism explained by an intrinsic illness of the pituitary gland (neoplasia, ischemia, infectious of infiltrative diseases, genetic syndromes, and immunological or inflammatory pathologies) and secondary hypopituitarism due to pituitary stalk, hypothalamus, or other central nervous systems (tumoral, infiltrative, traumatic, infectious, and nutritional) disorders [40]. The anterior pituitary part, known as adenohypophysis, secretes into the systemic circulation of the following hormones: thyroid-stimulating hormone (TSH), gonadotropins, somatotropin or growth hormone (GH), corticotropin or adrenocorticotropic hormone (ACTH), and prolactin.

Increased prevalence of metabolic syndrome and NAFLD was described in hypopituitarism, with cardiovascular disease notably higher, and significant premature mortality due to cardiovascular disease [41, 42]. From the classical point of view, this association between hypopituitarism, metabolic syndrome, and NAFLD is linked to lipid disequilibrium and liver fat accumulation. In hypopituitarism, there is a decreased high-density lipoprotein cholesterol level and an increased low-density/high-density lipoprotein ratio [42]. Thus, metabolic syndrome in patients with hypopituitarism is specifically distinguished by lower high-density lipoprotein level [43]. More recent arguments implicate leptin, an anorectic hormone, and the leptin resistance in hypopituitarism, in the pathogenesis of NAFLD, via insulin resistance, hyperphagia, and obesity [44, 45]. In an observational study including 69 patients with hypopituitarism without hormonal replacement therapy, the prevalence of ultrasound-diagnosed NAFLD was 77% compared to 12% in controls [46]. One cohort cross-sectional study showed that nontreated female patients with hypopituitarism have a double risk for cardiovascular mortality compared to the general population [42]. Hypopituitarism may be a rare cause of rapidly progressive NAFLD, as shown by a study reporting cases of young patients with fast deterioration towards cirrhosis in conjunction with hypopituitarism [47]. Another recent retrospective study including surgical and nonsurgical hypopituitarism patients revealed that liver fibrosis grade had a rapid increase in surgery cases of NAFLD patients with hypopituitarism, which correlated significantly with leptin serum levels [48]. Further studies are needed in order to completely describe the NAFLD and hypopituitarism relation and enforce timely therapeutic measures.

5. NAFLD and Growth Hormone Deficiency

Growth hormone (GH) is under the control of the hypothalamic-pituitary somatotropic axis, with an important metabolic role in the liver, adipose tissue, and skeletal muscle by regulating glucose and lipid metabolism, body composition, and growth in both children and adults [49]. Therefore, GH acts in

different tissues through complex mechanisms, either directly by interaction with the GH receptor or indirectly through its mediator insulin-like growth factor-1 (IGF-1) [50].

Growth hormone deficiency (GHD) is a rare disorder characterized by the inadequate secretion of GH from the anterior pituitary gland [1]. GHD can be congenital, due to genetic mutations or structural defects of the brain, acquired, secondary to a tumor, trauma, infection or radiation therapy, or idiopathic. GHD with childhood onset is manifested by growth retardation and short stature inconsistent with child's chronological age. GHD with adult onset is most commonly secondary to brain trauma or a pituitary tumor but can also be idiopathic. It is manifested by reduced muscular endurance, lipid abnormalities, insulin resistance, and impaired cardiac function [1].

The role of GH in the liver is known to stimulate gluconeogenesis and glycogenolysis, contrasting insulin signalling. Kim and Park demonstrated, in a recent study, that GHD causes insulin resistance, probably due to an increased flow of free fatty acids and the inhibition of glycogen synthesis, but the exact mechanisms remain unknown [51].

Recent studies have shown that adult patients with untreated GHD have a phenotype similar to that of the metabolic syndrome, which is strongly associated with NAFLD [49, 51], suggesting a possible association between GHD and NAFLD [52]. Based on this hypothesis, numerous studies have consistently shown that patients with NAFLD associate lower levels of serum GH compared to controls without NAFLD [53, 54]. At the same time, a correlation was noticed between lower GH levels and histological severity of NAFLD [54, 55]. A worrying fact is that GHD is a risk factor in the development and progression of secondary forms of NAFLD, which are not reversible on account of lifestyle changes [56, 57].

In a retrospective study, Adams et al. demonstrated a prevalence of NAFLD of approximately 2% in patients with hypothalamic or pituitary disorders [58]. More specifically, in patients with GHD, a 6.4-fold increased prevalence of NAFLD was found compared to age, sex, and appropriate BMI controls [46]. Ichikawa et al. demonstrated that patients with GHD had a significantly increased risk of NAFLD compared with those with pituitary dysfunction without GHD [59]. Also, Hong et al. in a case-control study showed that serum GH levels were lower in patients with NAFLD and the prevalence of NAFLD was significantly higher in men with hypopituitarism than in healthy controls [60].

Given the metabolic effects of GH on adipose tissue and liver, the main pathophysiological mechanism linking GHD to NAFLD is insulin resistance and increased lipogenesis, common pathways found in the development of NAFLD [1]. In addition, NAFLD development and progression in patients with GHD is also explained by the increased level of proinflammatory cytokines in the context of the systemic inflammatory status [1].

Therefore, based on the reported data, screening for NAFLD is recommended in all patients with GHD. Although study results are discrepant, given the beneficial effects on insulin resistance, inflammation, and fibrosis, the administration of exogenous GH for the treatment of secondary GHD NAFLD is justified [57, 61].

6. NAFLD and Hypogonadism

Hypogonadism is a congenital or acquired condition characterized by decreased reproductive function in both men and women, regardless of the cause [1]. Hypogonadism can be primary or secondary. Primary or hypergonadotropic hypogonadism (peripheral, gonadal) consists in an inadequate gonads response to gonadotropins resulting in decreased sex hormone levels and increased gonadotropin levels (luteinizing hormone, LH, and follicle-stimulating hormone (FSH)). Secondary or hypogonadotropic (central) hypogonadism refers to the inability of the hypothalamus or pituitary gland to produce enough FSH and LH and is characterized by low concentrations of sex hormones, FSH, and LH [62].

The main causes of primary hypogonadism are autoimmune diseases, genetic disorders, infections, liver and kidney disease, radiation, surgery, and trauma. Causes of central hypogonadism include pituitary hemorrhage, anorexia nervosa, radiation, medications (glucocorticoids, opiates), iron excess, surgery, trauma, and tumors [62].

Recently, numerous studies have reported a strong bidirectional association between NAFLD and hypogonadism in both genders [63–65]. Seo et al. demonstrated in a cross-sectional study on 1944 men without alcohol consumption that serum testosterone levels in NAFLD patients were reduced compared to non-NAFLD. At the same time, the higher the risk of NAFLD, the lower the testosterone level [66]. Moreover, Gild et al. demonstrated in a cohort of 380669 patients an improvement in NAFLD status in hypogonadic men who received hormone replacement therapy [64]. They also showed that there is a higher risk of NAFLD in men with prostate cancer and hypogonadism induced by androgen deprivation therapy [64]. Men with hypogonadism have higher noninvasive indices of NAFLD (hepatic steatosis index and triglyceride-to-HDL-C ratio), compared to men with high testosterone levels [67].

Also, in women with hypogonadism, an increased prevalence of serum liver enzymes has been demonstrated, surrogating indices of NAFLD [68, 69]. Also, it is known that estrogen deficiency, which can occur in hypogonadism or in postmenopausal women, is associated with a higher prevalence of NAFLD and advanced liver fibrosis. Prolonged estrogen deficiency has been associated with a long-term risk of advanced liver fibrosis [65].

The main pathophysiological mechanisms linking hypogonadism to NAFLD are complex and still under investigation. The main factors involved are estrogen deficiency [70], intestinal dysbiosis which causes a decrease in androgen hormones [71], and low levels of dehydroepiandrosterone, a hormone that affects insulin resistance and fibrogenesis [72] and adiposity, which is dependent on sex hormones and increases the risk of NAFLD development and progression through decreasing glucose tolerance [73].

General therapeutic measures are based on lifestyle changes, through dietary restrictions and regular exercise. Specific treatment measures depend on gender, so that in male hypogonadism, the treatment of choice is testosterone administration, while in women, hypogonadism is treated with estrogen replacement therapy [1].

In conclusion, it is recommended that patients with hypogonadism and NAFLD be monitored by both endocrinologist and hepatologist in order to prevent both the progression of liver damage as well as extrahepatic complications of NAFLD [74].

7. NAFLD and Hypercortisolism

Glucocorticoids (GCs) are produced by the adrenal gland under the control of pituitary ACTH secretion. High levels of circulating glucocorticoids favour liver gluconeogenesis and lower insulin sensitivity and are correlated with visceral obesity, dyslipidaemia, diabetes, arterial hypertension, coronary artery disease, and NAFLD [75]. Because of the similar metabolic disturbances and clinical features, there is an apparent overlap between metabolic syndrome and Cushing syndrome. However, the pathogenic pathway of GCs towards metabolic syndrome is still incompletely established. Excess tissue GCs are incriminated in the genesis of a metabolic syndrome rather than increased plasmatic GCs levels, based on the fact that circulating plasma corticosterone levels as well as urinary corticosterone levels is usually normal in patients with obesity and type 2 diabetes [76]. Active corticosterone (cortisol) becomes available from inert cortisone via specific prereceptor enzyme 11β-hydroxysteroid dehydrogenase-1 in key metabolic target tissues such as visceral adipose tissue and liver; the components of the metabolic syndrome may be a consequence of the increase in locally available glucocorticoids and not of the high circulating corticoid levels [77]. In truth, only 20% of patients with Cushing's syndrome have liver steatosis, measured by liver/spleen attenuation on CT scanning [78]. Conversely, even if it has been proved that NAFLD patients have hormonal abnormalities such as higher urinary free cortisol concentrations and lower dexamethasone suppression of plasma cortisol, there is no evidence to sustain an associated Cushing-like syndrome [79].

GCs' metabolism has an adaptive power since there are differences between uncomplicated steatosis and steatohepatitis; in simple steatosis, there is an increased clearance of cortisol as a protective mechanism trying to restrict lipid accumulation while, in steatohepatitis, the 11β-HSD1 has an increased activity in order to limit hepatic inflammation [80].

Base on the strength of its promoting role in lipogenesis, deletion or pharmacological inhibition of 11β-HSD1 could decrease hepatic steatosis [81], as favourable results on lipid profile and glycemic control are documented in patients with diabetes [82]. Even though there are clear associations between hypothalamic-pituitary-adrenal (HPA) axis disorders and NAFLD, further clarifications are needed especially in terms of targeted therapeutical directions.

8. Conclusions

The expanding prevalence of NAFLD and its relationships with many endocrine diseases are real challenges for clinicians nowadays. NAFLD has a potential for evolving towards severe complications such as cirrhosis or hepatocellular carcinoma but may also expose to various extrahepatic manifestations, adding to the burden weighing on patients and practicing physicians alike. Irrespective of the fact that NAFLD patients are at risk of developing specific endocrine pathologies or, contrarily, some endocrinopathies predispose patients at risk of developing NAFLD, awareness about the interconnections between NAFLD and endocrinopathies is of utmost importance, in order to provide to all patients appropriate surveillance and optimal therapeutical approach.

Data Availability

The data used to support this study are included within this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors contributed equally to this review. All authors have read and agreed on the published version of the manuscript.

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Review Article

Nonalcoholic Fatty Liver Disease-Related Chronic Kidney Disease

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Nonalcoholic fatty liver disease has become the main concern of hepatologists around the world and the main research topic for identifying effective and safe therapy. Advances in the treatment of chronic viral hepatitis in recent years have opened the way towards reducing mortality in patients with chronic liver disease. This goal has not yet been reached, as the burden of chronic liver disease remains a future major health problem as the incidence of the nonalcoholic fatty liver disease continues to rise. The proportion of patients with liver cirrhosis and those with hepatocellular carcinoma due to nonalcoholic liver disease on the liver transplant waiting list has increased in the last years. The upward trend in the incidence and prevalence of the disease in recent decades raises concern over a possible global epidemic, especially as the disease is still underestimated and underdiagnosed. Chronic kidney disease presented an increase in incidence and prevalence during the last years, and it has been associated not only with increased morbidity and mortality but also with high costs for the health system. During the last decade, several studies have shown the association between nonalcoholic fatty disease and chronic kidney disease, two major worldwide health problems.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) and chronic kidney disease (CKD) are worldwide public health problems, due to their increasing prevalence, poor outcomes, and health care burden [1–5]. Nonalcoholic fatty liver disease is the most common etiology of chronic liver disease worldwide, especially in developing countries.

NAFLD covers a wide range of diseases from benign steatosis (fat accumulation in >5% of hepatocytes, especially macrovesicular, without inflammation or fibrosis) to nonalcoholic steatohepatitis (NASH) which is characterized by liver inflammation with high potential to progress to advanced fibrosis, liver cirrhosis, and hepatocellular carcinoma.

In a recent analysis of the Third National Health and Nutrition Survey database (including ~11,700 American subjects), the moderate to advanced stages of CKD in patients with ultrasound-detected NAFLD were independently associated with increased all-cause mortality over a mean follow-up period of 19 years [6].

During the last decade, several studies have shown the association between NAFLD and CKD, regardless of the presence or not of known risk factors for diseases such as obesity, hypertension, type 2 diabetes mellitus (T2DM), or metabolic syndrome [7–10].

CKD has high rates in patients with NAFLD, ranging between 20% and 50% compared to 5% and 25% in those without [5, 11–14].

2. Chronic Kidney Disease: Definition and Staging

CKD is defined as either decreased estimated glomerular filtration rate (eGFR) (<60 ml/min/1.73^2) and/or abnormal albuminuria and/or overt proteinuria for 3 or more months [5, 7, 15, 16]. Kidney failure is defined as an eGFR of less than 15 mL/min per 1.73 m^2 or the need for treatment with

dialysis/transplantation [15]. The most common tests for CKD diagnosis include eGFR and urinary albumin-to-creatinine ratio (ACR: normal value, <30 mg/g), the essentially diagnostic tools used in classification of CKD patients into five stages [5, 7]. Based on GFR levels, CKD is classified into five stages: stage 1, GFR more than 90 mL/min per 1.73 m^2; stage 2, 60–89 mL/min per 1.73 m^2; stage 3, 30–59 mL/min per 1.73 m^2 (stage 3a, 45–59 mL/min per 1.73 m^2; stage 3b, 30–44 mL/min per 1.73 m^2); stage 4, 15–29 mL/min per 1.73 m^2; and stage 5, GFR less than 15 mL/min per 1.73 m^2 [16, 17].

3. NAFLD and CKD: Epidemiological Data

A large meta-analysis including nearly 64,000 participants in 20 cross-sectional studies and 13 longitudinal studies showed that NAFLD was associated with an approximately 2-fold increase in both prevalence (OR, 2.12; 95% CI, 1.69 to 2.66) and incidence of CKD (HR, 1.79; 95% CI, 1.65 to 1.95) [17]. The NAFLD was most often assessed using noninvasive methods (scoring systems for fibrosis evaluation and ultrasonography) and only in a few cases by liver biopsy.

Some data suggest that the degree of renal impairment is correlated with the histological severity of NASH, the progressive type of NAFLD, and the hepatic fibrosis stage [14]. In the same meta-analysis, NASH was associated with a higher prevalence and incidence of CKD than simple hepatic steatosis, and advanced fibrosis was associated with a higher prevalence (OR, 5.20; 95% CI, 3.14 to 8.61) and incidence (HR, 3.29; 95% CI, 2.30 to 4.71) of CKD than nonadvanced fibrosis [17]. An Italian study on 570 White subjects found that patients with high risk for developing liver fibrosis had a 5.1-fold increased risk of developing CKD compared with low-risk patients (OR: 5.1; 95% CI = 1.13–23.28; $p = 0.03$), while intermediate-risk subjects had a 3-fold increased risk of developing liver fibrosis and had 3 times increased risk of developing CKD compared to low-risk patients (OR: 3.01, 95% CI = 0.87–10.32; $p = 0.07$) [18].

In a recent longitudinal study, Jang et al. showed that NAFLD is an independent risk factor associated with the progression of CKD. The risk of CKD progression was higher in patients with advanced NAFLD (probably associated with a significant/advanced hepatic fibrosis score) and in those with lower eGFR with/without proteinuria [19].

Similar results were reported by Mantovani in a systematic review and meta-analysis (9 observational cohort studies including approximately 96,500 middle-aged individuals of predominantly Asian descent, over a median follow-up period of 5.2 years). The authors found a 40% increase in the long-term risk of incident CKD (random-effect HR, 1.37; 95% CI, 1.20–1.53; $I^2 = 33.5\%$) correlated with the severity of liver fibrosis; they showed that the risk of CKD in NAFLD patients remained significant even after adjustment for age, sex, obesity, hypertension, smoking, T2DM, baseline eGFR, or medications [20].

Few data suggest that lifestyle modification over a year in patients with biopsy-proven NASH leads to the histologic resolution of NASH and improvement in liver fibrosis stage independently associated with improvement in renal function (increase in eGFR values) [21].

Most of the cohort studies included in meta-analyses are reported in Asian countries, where large populations undergo regular health check-up programs, besides having different genetic, dietary factors, and adipose tissue distributions [5]. The discrepancy between eastern and western populations regarding the NAFLD-CKD relationship has been found in previous studies. While the National Health and Nutrition Examination Survey (NHANES) study of 11,469 US adults showed no increased risk of CKD in patients with ultrasonography diagnosed NAFLD, after correcting for the presence of the metabolic syndrome [12], a large prospective cohort study of 8329 Korean men without T2DM, hypertension, or CKD at baseline followed up for 4 years showed that patients with NAFLD had a significantly higher risk of developing CKD, after correcting for the same risk factors [9, 22].

The relationship between NAFLD and CKD is still poorly understood, and the mechanism relating NAFLD with renal dysfunction to date is yet unknown. Moreover, as in patients with T2DM, the link between NAFLD and CKD is bidirectional, so that kidney damage *per* se subsequently contributes to the progression of liver damage [23].

Many studies suggest that NAFLD and CKD share common pathogenetic mechanisms: oxidative stress, impaired regulation of the renin-angiotensin system, and alterations in the gut microbiota [24].

The currently available data suggest that NAFLD could be a driver force for the development and progression of CKD, rather than a marker of CKD [5].

4. NAFLD and CKD: Clinical Approach

In clinical practice, renal function should be evaluated and monitored in all patients with NAFLD as in patients with liver cirrhosis. In a large meta-analysis, Musso et al. suggest that patients with NAFLD should be screened for CKD even in the absence of other classical risk factors, and this is especially recommended if NASH and/or advanced fibrosis are suspected [25].

Although there are no guidelines, surveillance protocols for CKD in patients with NAFLD, it is crucial to detect early renal impairment in these patients to prevent CKD progression, minimize complications, and improve survival [7, 26].

Clinicians should therefore identify CKD stage ≤3; all CKD above stage 3 (abnormal albuminuria (ACR >−30 mg/g) or overt proteinuria, urine sediment abnormalities, and eGFR <60 ml/min/1.73 m^2 are associated with a high or very high risk of disease progression [5]. Armstrong et al. proposed that the annual screening in patients with NAFLD for CKD by eGFR and microalbuminuria could detect early renal impairment in patients with NAFLD [27], and we consider that, in all new patients diagnosed with NAFLD, the renal function assessment is mandatory, and all medications that could affect kidney function in patients with NAFLD must be evaluated to allow drug-dosage adjustment [28].

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

All authors contributed equally to this review. All authors have read and agreed to the published version of the manuscript.

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Review Article

Nonalcoholic Fatty Liver Disease and Type 2 Diabetes Mellitus: A Bidirectional Relationship

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Worldwide, the leading cause of chronic liver disease is represented by nonalcoholic fatty liver disease (NAFLD) which has now become a global epidemic of the 21st century, affecting 1 in 4 adults, and which appears to be associated with the steadily increasing rates of metabolic syndrome and its components (obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia). NAFLD has been reported to be associated with extrahepatic manifestations such as cardiovascular disease, T2DM, chronic kidney disease, extrahepatic malignancies (e.g., colorectal cancer), endocrine diseases (e.g., hypothyroidism, polycystic ovarian syndrome, psoriasis, and osteoporosis), obstructive sleep apnea, and iron overload. The prevalence of NAFLD is very high, affecting 25–30% of the world population and encloses two steps: (1) nonalcoholic fatty liver (NAFL), which includes steatosis only, and (2) nonalcoholic steatohepatitis (NASH) defined by the presence of steatosis and inflammation with hepatocyte ballooning, with or without fibrosis which can progress to liver fibrosis, hepatocellular carcinoma, and liver transplantation. Current data define a more complex relationship between NAFLD and T2DM than was previously believed, underlining a bidirectional and mutual association between the two entities. This review aims to summarize the current literature regarding the incidence of T2DM among patients with NAFLD and also the prevalence of NAFLD in T2DM patients, highlighting the recent key studies. Clinicians should screen, diagnose, and treat T2DM in patients with NAFLD in order to avoid short- and long-term complications.

1. Introduction

The incidence rate of chronic liver diseases increased through the years with a worrying rise of liver-related morbidity and mortality rates worldwide [1]. One of the major causes of chronic liver diseases is represented by nonalcoholic fatty liver disease (NAFLD) which has now become a global epidemic affecting 1 in 4 adults, with an estimated prevalence between 25% and 30%, and appears to be associated with the steadily increasing rates of metabolic syndrome (MetS) and its components (obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia) [1–3]. The mandatory feature of NAFLD is the presence of liver steatosis (LS) in the absence of other causes of chronic liver disease [4]. Although initially NAFLD was considered as the hepatic manifestation of MetS, there is now clear evidence that NAFLD is a key driver in MetS and hepatic involvement is only one component of systemic multiorgan involvement [5, 6]. NAFLD encloses two distinct conditions with different histologic features and prognosis: (1) nonalcoholic fatty liver (NAFL), which includes steatosis only, and (2) nonalcoholic steatohepatitis (NASH) characterized by steatosis and inflammation with hepatocyte ballooning, with or without fibrosis, which can progress to liver fibrosis (LF), cirrhosis, hepatocellular carcinoma (HCC), and liver transplantation [7]. The prevalence of NASH among

NAFLD patients ranges from 10% to 59% in patients who underwent liver biopsy [1], meaning that millions and millions of people worldwide are at risk of cirrhosis and its complications. Even more alarming is that the continuously increasing rates of MetS and its components parallel the rising prevalence of NASH, with obesity, T2DM, and MetS being the most important risk factors [8, 9].

Growing evidence clearly shows that NAFLD is a multiorgan disease, supporting a strong link between NAFLD and cardiovascular diseases (CVDs), T2DM, chronic kidney disease (CKD), extrahepatic malignancies (eg., colorectal cancer), obstructive sleep apnea (OSA), and various endocrinopathies (e.g., thyroid dysfunction, polycystic ovarian syndrome (PCOS), osteoporosis, psoriasis, hypothyroidism, and iron overload) [10, 11]. Although the primary site of NAFLD is the liver, the most common causes of mortality are CVDs, followed by extrahepatic malignancies such as colorectal cancer and then liver-related complications (cirrhosis and HCC) [5, 6, 12]. Considering the high clinical and economic burden of NAFLD, the main point in the management of these patients is an early acknowledgement of both hepatic and extrahepatic manifestations and their subsequent complications [11, 13, 14]. Current data outline a more complex relationship between NAFLD and T2DM than was previously thought, pointing out a bidirectional and mutual association between the two entities. Thus, clinicians should screen, diagnose, and treat T2DM in patients with NAFLD in order to avoid short- and long-term complications. Herein, this review aims to summarize the current literature regarding the incidence of T2DM among patients with NAFLD and also the prevalence of NAFLD in T2DM patients, highlighting recent key studies.

2. Relation between NAFLD and T2DM

Current data reveal a more complex relationship between NAFLD and T2DM than was previously believed, highlighting a bidirectional and mutual association between the two entities [11, 15, 16]. Considering that NAFLD and T2DM have similar physiopathological pathways, one can precede and/or promote the other [10, 17].

2.1. T2DM in Patients with NAFLD. NAFLD is associated with lipotoxicity which is secondary to the accumulation of triglyceride-derived toxic metabolites in the liver, pancreas, and muscles, which leads to the activation of the inflammation cascade and insulin resistance [18, 19]. The hepatic insulin resistance associated with NAFLD is the key driver for the development of T2DM among these patients.

The prevalence of T2DM in patients with NAFLD depends on the severity of NAFLD starting from 9.8% in mild NAFLD to 17.8% in moderate to severe NAFLD [20–22]. Although NAFLD is considered an independent risk factor for developing T2DM, with a 2-fold incidence increase in these individuals, patients with NASH have an up to threefold higher risk of developing incident T2DM compared with those with simple steatosis [20, 23, 24].

Despite evidence from several studies which demonstrated that high levels of NAFLD's surrogate markers—gamma-glutamyl transferase and alanine aminotransferase (ALT)—were associated with a high incidence of T2DM, the predictive value of these biologic parameters is limited due to the possibility of normal levels among these patients [25–29]. As for ultrasonography-defined NAFLD (liver steatosis ≥20%) [30, 31], recent data showed a twofold to fivefold increased risk of T2DM [32].

Many large-population-based retrospective studies with a follow-up period of 3 to 6.2 years showed an increased incidence risk of T2DM among patients diagnosed with NAFLD (Table 1) [33–37, 42, 43]. Shibata et al. achieved a 4-year follow-up in 3189 patients among whom 1138 had NAFLD at baseline and reported an incidence of 1.8% vs. 8.1% in the non-NAFLD patients compared with those with NAFLD [33]. Similarly, 4 other retrospective studies which included Asian cohorts without T2DM, demonstrated that NAFLD was significantly associated with a high incidence of T2DM during follow-up [34–37]. In a similar manner, prospective studies which evaluated the prevalence of T2DM among patients with NAFLD have also reported an increased risk associated with baseline NAFLD [38–41]. Results from an Israeli cohort of 141 nondiabetic participants among which 24.8% had NAFLD, followed up for an average period of 6.08 ± 0.7 years, demonstrated a higher incidence of prediabetes and/or T2DM in patients with NAFLD than in subjects without NAFLD (74.3% vs. 48.1%) [39]. In a recent retrospective study conducted by Liu et al. which included 18,507 nondiabetic subjects, the prevalence of NAFLD was 18.77% with a 5-year T2DM incidence of 2.44%. The authors concluded that patients with baseline NAFLD had a higher risk incidence of T2DM, with an adjusted relative risk of 1.672 [43].

2.2. NAFLD in Patients with T2DM. Once established, T2DM may promote the progression to NASH and become an independent risk factor for chronic liver disease, including cirrhosis and HCC [10]. It has been reported that the presence of NAFLD among patients with T2DM determined a 2.2-fold risk increase in all-cause mortality, compared with those without NAFLD [15]. The estimated prevalence of NAFLD among patients with T2DM is about 75%, which is more than the prevalence in the general population [1].

Evidences from several recent cohort studies highlighted an increased incidence of NAFLD among patients diagnosed with T2DM, with an estimated prevalence ranging between 41.6% and 86% (Table 2) [8, 44–49]. For instance, Sporea et al. conducted a prospective study in which 534 patients diagnosed with T2DM were included. Patients with other causes of steatosis were excluded, and LF and steatosis were quantitatively assessed using vibration controlled transient elastography and the controlled attenuation parameter. The authors found an estimated prevalence of NAFLD of 76.1%. Moreover, severe steatosis was detected in 60.3%, while advanced LF had a prevalence of almost 20% [50]. Two other retrospective studies reported a similar prevalence of NAFLD among T2DM patients [45, 46].

Table 1: Studies which assessed T2DM incidence among patients with NAFLD.

Ref.	Study design and period of surveillance	NAFLD assessment	Cases with T2DM; % in NAFLD vs. non-NAFLD
Shibata et al. 2007 [33]	Retrospective cohort study; $n = 3,189$ (33.6% with NAFLD); male Japanese; 4 years	Liver ultrasonography	$n = 109$ incident cases; 1.8% vs. 8.1%
Kim et al. 2008 [34]	Retrospective cohort study; $n = 5,372$ (33.3% with NAFLD); South Korean subjects without diabetes; 5 years	Liver ultrasonography	$n = 234$ incident cases; 2.3% vs. 8.5%
Bae et al. 2011 [35]	Retrospective study; $n = 7,849$ (29.2% with NAFLD); subjects without diabetes; 5 years	Liver ultrasonography	$n = 435$ incident cases; 3.7% vs. 9.9%
Sung et al. 2012 [36]	Retrospective cohort study; $n = 12,853$ (27.6% with NAFLD); subjects without diabetes; 5 years	Liver ultrasonography	$n = 223$ incident cases; 0.8% vs. 4.3%
Kasturirante et al. 2013 [37]	Retrospective cohort study; $n = 2,276$ (40.7% with NAFLD); individuals without diabetes; 3 years	Liver ultrasonography	$n = 242$ incident cases; 10.5% vs. 19.7%
Park et al. 2013 [38]	Prospective cohort study; $n = 25,232$ (35% with NAFLD); men without diabetes; 5 years	Liver ultrasonography	$n = 2,108$ incident cases; 7% in no steatosis group vs. 17.8% in moderate to severe steatosis group
Zelber-sagi et al. 2013 [39]	Prospective cohort study, $n = 213$; without known liver disease and alcohol abuse; 7 years	Liver ultrasonography	$n = 106$ incident cases with NAFLD
Chen et al. 2016 [40]	Prospective cohort study; $n = 6,542$; Chinese subjects without diabetes; 6 years	Liver ultrasonography	$n = 368$ incident cases
Li et al. 2017 [41]	Prospective cohort study; $n = 18,111$ (31.8% with NAFLD); Chinese subjects without diabetes without known chronic liver diseases; 4.6 years follow-up	Liver ultrasonography	$n = 1,262$ incident cases; 4.6% in non-NAFLD group vs. 18.1 in moderate to severe NAFLD group
Ma et al. 2017 [42]	Retrospective cohort study; $n = 1,051$ (17.8% with NAFLD); US individuals without diabetes without known chronic liver diseases; 6.2 years	Liver tomography and ultrasonography	$n = 64$ incident cases
Liu et al. 2017 [43]	Retrospective study, $n = 18,507$; men without diabetes; 5 years	Liver ultrasonography	$n = 453$ incident cases

NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus.

Table 2: Studies which assessed NAFLD prevalence among patients with T2DM.

Ref.	Study design; follow-up; and population	Diagnostic method of NAFLD	Prevalence of NAFLD
Williamson et al. 2011 [8]	Retrospective study; $n = 918$; 1 years	Ultrasound	42,6%
Lv et al. 2013 [44]	Prospective cohort study; $n = 1217$; 4 years	Ultrasound	61%
Silaghi et al. 2015 [45]	Retrospective cohort study; $n = 336$; N/A	Ultrasound	86%
Mantovani et al. 2016 [46]	Retrospective cohort study; $n = 330$; 2 years	Ultrasound	72,1%
Guo et al. 2017 [47]	Prospective cohort study; $n = 8571$; 9 years	Ultrasound	50,6%
Yi et al. 2017 [48]	Prospective cohort study; $n = 3861$; 1 year	Ultrasound	45,4%
Ding et al. 2017 [49]	Prospective cohort study; $n = 1648$; 1 year	Ultrasound	41,6%
Sporea et al. 2020 [50]	Prospective cohort study; $n = 534$; N/A	Ultrasound	76,1%

NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; N/A, not available.

3. Management of Patients with NAFLD and T2DM

The reciprocal relationship between T2DM and NAFLD leads to the progression of LF and is secondary to the development of liver-related complications with high morbidity and mortality rates. In order to avoid systemic multicollateral damage, it seems appropriate to screen patients with NAFLD for T2DM, and vice-versa. According to current European Guidelines, screening of T2DM in patients with NAFLD is mandatory, and it consists of random blood glucose or hemoglobin A1C [51]. Furthermore, screening for NAFLD and LF is recommended by the American Diabetes Association in patients diagnosed with T2DM which have elevated liver enzymes (ALT) or LS, while the American Association Society of Liver Disease is not in favour of routine screening in these patients [52, 53].

4. Conclusion

In light of current evidence, the clinical burden of NAFLD is not limited to liver-related complications, but is in fact, related to its extrahepatic manifestations such as CVD, T2DM, CKD, extrahepatic malignancies, OSA, and various endocrinopathies, with increased mortality rates. Our review highlights that the components of NAFLD (NASH and NAFL) lead to insulin resistance and T2DM through various physiopathological pathways, but also T2DM may promote the progression to NASH as an independent risk factor. Thus, clinicians should be aware of these NAFLD-related extrahepatic manifestations considering that an early acknowledgement of T2DM is the key point in the management of these patients.

Conflicts of Interest

The authors declare no conflicts of interest in this work.

Authors' Contributions

All authors made substantial contributions to acquisition of data, or analysis, conception and design, and interpretation of data; took part in drafting of the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

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Research Article

The Clinical Significance of Colon Polyp Pathology in Nonalcoholic Fatty Liver Disease (NAFLD) and Its Impact on Screening Colonoscopy in Daily Practice

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Aim. Nonalcoholic fatty liver disease (NAFLD) has been known as a risk for the presence of colon polyp and CRC development. This study was aimed to find out the clinical significance of colon polyps' pathology among NAFLD patients. *Method*. A retrospective database study was done in patients who underwent elective colonoscopy within one-year period in a referral private hospital, Jakarta. Subjects were adult patients who also had documented abdominal ultrasound (US). The association between NAFLD and colonic polyp was analyzed using Chi-square test with odds ratio (OR) and its corresponding 95% confidence interval (CI). *Results*. A total of 138 adult patients were enrolled; 68 (51.1%) were men. Patients' mean age was 56.8 ± 15.3 years old. Colon polyps were found in 49 (35.5%) cases; the most common histopathology was adenoma (42.9%). NAFLD was found in 68 (49.3%) of patients. Colon polyps were found to be more among patients with NAFLD than in those without NAFLD (44.1% vs. 27.1%; OR: 2.119; 95% CI: 1.040–4.318). Colon polyps were found in 30 (44.1%) NAFLD patients, where 18 (26.5%) patients had adenomatous polyp, and from this subset of patients with adenomatous polyp, 6 (8.8%) patients had mild dysplasia, 8 (11.8%) had moderate dysplasia, 1 (1.5%) had severe dysplasia, and 3 (4.4%) had adenocarcinoma. *Conclusions*. NAFLD is associated with increased risk of any colon polyp, regardless of the histopathological type, compared with patients without NAFLD. This finding implies the necessity to perform screening colonoscopy in patients with NAFLD in the future.

1. Background

Colorectal cancer (CRC) is still one of the most common cancers in the Western and Asian countries. Until now, the general recommendation for CRC screening is still based on invasive diagnostic tests, such as colonoscopy, and noninvasive tests, such as Asia-Pacific colorectal screening score (APCS) and fecal immunochemical test (FIT) [1, 2]. However, the patient's reluctance for invasive screening tests, different subgroups of patient's diversity, different screening program policies in every country, especially in developed and developing Asian countries, cost difference, and availability of diagnostic tools make a different strategy approach in the real clinical practice.

Nonalcoholic fatty liver disease (NAFLD) has been known to be associated with extrahepatic causes of death, including CRC [3]. Early studies in Asia have observed an increase of CRC risk in patients with ultrasound-diagnosed

NAFLD compared with controls [4, 5]. The presence of NAFLD was found as an independent risk factor for colorectal adenomatous polyps in asymptomatic subjects who underwent routine colonoscopy [6, 7]. A similar finding was also reported in Western population [8]. A systematic review involving 6263 asymptomatic subjects undergoing screening colonoscopy confirmed the association between colorectal adenoma and NAFLD [9].

A large prospective cross-sectional study in a referral private hospital in Indonesia showed that NAFLD is a common finding in unselected adult patients who underwent routine medical check-up. Several risk factors were confirmed as independent risk factors for NAFLD, that is, obesity, male gender, age of more than 35 years, high triglyceride, low high-density lipoprotein (HDL) cholesterol, and high serum alanine aminotransferase levels [10]. Notwithstanding, retrospective data analysis study of a large sample size in the same private hospital for colon polyps or cancer detection rate found almost similar important risk factors, that is, older age (≥50 years old) and male gender [11].

However, until now, there is no consensus about how the CRC screening should be done in NAFLD population. Therefore, considering the increased prevalence of NAFLD in younger age, possible genetic and environment differences, different CRC molecular pathways, and different conclusion of pathologist's specimen evaluation, the author would like to highlight this issue based on the pathology finding of colon polyp in NAFLD patients.

2. Method

2.1. Study Design and Subjects. A retrospective database study was done in unselected patients who underwent elective colonoscopy within one-year period in Medistra Hospital, Jakarta. Data was collected on the patients' demography and colonoscopy findings including the presence of hemorrhoids, polyps, diverticula, inflammation, or tumor mass. Patients were included if they also had a transabdominal ultrasound study record, as it is the preferred first-line diagnostic imaging procedure for imaging screening of NAFLD [12]. Patients with evidence of hepatitis virus infection, autoimmune hepatitis, significant alcohol consumption, history of any other cancers, or familial adenomatous polyposis (FAP) were excluded.

2.2. Colonoscopy Procedure. Following full bowel preparation, a conventional colonoscopy procedure was performed in all patients using adult high-definition video fiber optic colonoscopy with an auxiliary water jet (CFQ160AL, Olympus, USA). Polyps were removed by snare-loop polypectomy at the bottom of the stalk or by biopsy forceps. Flat or sessile polyps were removed by performing a submucosal lifting technique with the injection of saline solution into the submucosal layer. Polypectomy was not performed in patients with known absolute contraindications such as anticoagulant therapy and bleeding disorders.

2.3. Histopathological Diagnosis. Biopsy specimens were evaluated and classified according to the World Health Organization (WHO) Classification by two board-certified pathologists who were blinded to clinical data. High-grade dysplasia or in situ carcinoma is defined by the considerable loss of nuclear polarity with irregular glandular architecture with no involvement beyond the muscularis mucosa. Submucosal invasive early colorectal cancer was defined as malignant lesions that invade the submucous layer, while cancer was defined as invasion of malignant cells beyond the muscularis mucosa.

2.4. Statistical Analysis. Patients' demography was presented descriptively. Categorical data were compared using the Chi-square test. A p value of less than 0.05 was considered significant. Risk for polyp development in patients with NAFLD compared with patients without NAFLD was expressed as odds ratio (OR) and its corresponding 95% confidence interval (CI). Median difference was assessed using Mann–Whitney U test for skewed data. Statistical analysis was done using the SPSS software version 19.0 (SPSS Inc., Chicago, Illinois, USA).

3. Results

Four hundred and fourteen patients underwent elective colonoscopy during the study period; however, only 138 patients had documented of transabdominal ultrasound results. Seventy-two patients (52.2%) were men and their mean age was 56.8 ± 15.3 years old. NAFLD was diagnosed in 68 (49.3%) of patients. Colon polyps were found in 49 (35.5%) patients (Table 1). The most common histopathology was adenoma and mostly having a mild dysplasia (Table 2).

Colon polyps were found among patients with NAFLD more than those without NAFLD (44.1% vs. 27.1%; $p = 0.037$). The presence of NAFLD was associated with 2.1 times increased risk of the presence of colon polyp (Table 3). The median age of patients with polyp tended to be higher than that of patients without polyp (59 vs. 56 years; $p = 0.639$, Mann–Whitney U test). Colon polyp was found in 30 (44.1%) NAFLD patients, where 18 (26.5%) patients had adenomatous polyp, and from this subset of patients with adenomatous polyp, 6 (8.8%) patients had mild dysplasia, 8 (11.8%) had moderate dysplasia, 1 (1.5%) had severe dysplasia, and 3 (4.4%) had adenocarcinoma (Table 4). Based on the polyps' location, 10 (33.3%) patients had a right-sided polyp, and 20 (66.7%) patients had a left-sided polyp (Table 5).

Based on dysplasia's type, there was no statistically significant difference between the presence of mild and moderate-to-severe grade of dysplasia in NAFLD patients (Table 6).

4. Discussion

The impact of colon polyps' pathology in NAFLD patients as a prevention for CRC development has not been well documented. Our current study in Indonesia, which

Table 1: Characteristics of the study subjects ($N = 138$).

Variable	n (%)
Age group	
<50 years	43 (31.2)
>50 years	95 (68.8)
Gender	
Male	72 (52.2)
Female	66 (47.8)
Presence of NAFLD	
Yes	68 (49.3)
No	70 (50.7)
Degree of NAFLD ($n = 68$)	
Mild	62 (91.2)
Moderate	5 (7.3)
Severe	1 (1.5)
Presence of polyp	
Yes	49 (35.5)
No	89 (64.5)

Table 2: Characteristics of the polyps ($n = 49$).

Characteristic	n (%)
Side of polyp ($n = 49$)	
Right	14 (28.6)
Left	35 (71.4)
Histopathology of polyp ($n = 49$)	
Tubular adenoma	21 (42.9)
Inflammatory polyp	7 (14.3)
Serrated adenoma	4 (8.2)
Tubulovillous adenoma	3 (6.1)
Hyperplastic polyp	2 (4.1)
Adenocarcinoma	6 (12.2)
Not biopsied	6 (12.2)
Grade of dysplasia ($n = 34$)	
Mild	16 (47.1)
Moderate	10 (7.2)
High	2 (1.4)
Malignant	6 (4.3)

Table 3: Associations among clinical variables and colon polyps ($n = 138$).

Variable	Polyp (+)	Polyp (−)	p value	OR	95% CI
Gender					
Male	27 (37.5)	45 (62.5)	0.609	1.200	0.596–2.416
Female	22 (33.3)	44 (66.7)			
Age group					
≤50 years	13 (30.2)	30 (69.8)	0.384	0.710	0.328–1.536
>50 years	36 (37.9)	59 (62.1)			
NAFLD					
Yes	30 (44.1)	38 (55.9)	**0.037**	**2.119**	**1.040–4.318**
No	19 (27.1)	51 (72.9)			

represents the biggest Southeast Asian country, is the first report showing an association between NAFLD and colorectal polyp. Generally, there are two responsible important factors known as the cause of the CRC development, such as genetic predisposition (FAP and HNPCC) and environmental factors; however, sporadic CRC has been noted as the most common pathway in CRC development [13]. Sporadic pathway is usually based on chromosomal instability (CIN)

Table 4: Association between NAFLD and polyp type ($n = 43$).

NAFLD	Adenoma	Nonadenoma	p value	OR	95% CI
Yes	18 (75.0)	6 (25.0)	0.708*	0.563	0.120–2.626
No	16 (84.2)	3 (15.8)			

*Fisher's exact test.

Table 5: Association between NAFLD and polyps' location ($n = 49$).

NAFLD	Right-sided polyp	Left-sided polyp	p value	OR	95% CI
Yes	10 (33.3)	20 (66.7)	0.354	1.875	0.491–7.153
No	4 (21.1)	15 (78.9)			

Table 6: Association between NAFLD and degree of adenoma dysplasia ($n = 34$).

NAFLD	Mild dysplasia	Higher dysplasia*	p value	OR	95% CI
Yes	12 (66.7)	6 (33.3)	0.089	3.333	0.815–13.637
No	6 (37.5)	10 (62.5)			

*Including moderate dysplasia, severe dysplasia, and malignant carcinoma.

or microsatellite instability (MSI). This pathway contributes to CRC development in patients with a median age of 70–75 years old. However, molecular CRC study in Indonesia showed that sporadic CRC was found in younger patients (40 years old or less) without any clear family history [14]. There has been an analysis about association between physical inactivity and CRC development [15]. Obesity is also considered as an independent risk factor for CRC development [16]. Our large prospective study has shown the significant association between high BMI and presence of NAFLD [11].

However, patients with NAFLD harbored a twofold increased risk of colon polyp, regardless of the histopathology findings. In comparison, other studies reported an increased risk of adenomatous polyp, with an OR ranging from 1.28 in patients with NAFLD [7] to 4.89 in patients with NASH, using multivariate analyses [6]. A recent meta-analysis demonstrated that NAFLD was associated with a high risk of colorectal adenoma and the number of polyps, but not with its location, size, and advanced nature [17]. Furthermore, a recent study found that advanced liver fibrosis significantly increases the risk of colorectal adenoma, advanced adenoma, and multiple adenomas [18].

In this study, colon polyps were found in 44.1% of NAFLD patients. Based on pathology point of view, adenoma (tubular and tubulovillous) and serrated adenoma were found in 50% of the patients with polyp. However, only 26.5% NAFLD patients had an adenomatous polyp. It is well known that adenomatous polyp is the most important polyp for CRC development. However, conventional adenomas (tubular, villous, and tubulovillous) are not the only precursor lesions of CRC. It is now recognized that CRC is not a single disease but more represents a constellation of heterogeneous subtypes that develop from different pathways [19]. Interestingly, our study also revealed early malignant transformation of the colon polyp in three NAFLD patients. The current WHO classification lists several precursor lesions of CRC, that is, adenomas, serrated lesions, chronic inflammatory bowed disease, and hamartomatous polyps (juvenile and Peutz-Jeghers polyps) [20]. Serrated lesions are a heterogeneous group that includes hyperplastic polyp, sessile serrated adenoma, and traditional serrated adenoma [21]. The earliest lesion in this group is nondysplastic aberrant crypt foci, which are considered as the precursor lesions of hyperplastic polyps [22]. Inflammatory polyps are generally nonneoplastic and are often related to inflammatory bowed disease or ischemic colitis [23]. However, we still included these polyps since their association with NAFLD has not been well established.

Since NAFLD and colon polyps share similar risk factors, patients with NAFLD might be targeted for CRC screening. Although most colon polyps are usually found in older age and elderly patients, about 25% of patients under 50 years old in our study presented with colon polyps. Considering that regular screening colonoscopy has been recommended for people aged 50 years and older [24], this finding raises the big question of whether colonoscopy should be done earlier, especially in patients with NAFLD. The recent guidelines from the American Cancer Society recommend initiating screening for CRC at the age of 45 years for all average-risk adults [25]. The recommendation to lower the starting age of screening is based on limited empirical data related to outcomes in average-risk individuals in this age group since the previous recommendation to screen at 50 years old has been largely based on expert opinion. However, the average-risk individuals were meant for persons without a history of adenomatous polyp or colorectal cancer or a family tendency of colorectal cancer.

This study has limitations due to retrospective database study. First, it still cannot establish a cause-and-effect relationship and cannot confirm that NAFLD is an independent risk factor of colorectal adenoma. However, these

findings have given a new insight into the prevention of CRC development in NAFLD patients. Second, the metabolic risk factors are not analyzed in this study. However, it is already well known that NAFLD is strongly related to metabolic risk factors. A larger and more comprehensive study addressing metabolic factors and colorectal polyps' pathology is needed to find stronger recommendation about screening colonoscopy in every NAFLD patients in clinical practice.

5. Conclusions

NAFLD is associated with increased risk of any colorectal polyp, regardless of the histopathological type, compared with patients without NAFLD. This finding implies the necessity to perform screening colonoscopy in patients with NAFLD. Further study is needed to assess other risk factors and patients' eligibility to undergo full colonoscopy when NAFLD is found during routine medical check-up.

Data Availability

The database belongs to the Endoscopy Unit of Medistra Hospital, Jakarta.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Acute Coronary Syndromes and Nonalcoholic Fatty Liver Disease: "Un Affaire de Coeur"

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Background and Aims. Both nonalcoholic fatty liver disease (NAFLD) and ischemic heart disease have common pathogenic links. Evidence for the association of NAFLD with acute coronary syndromes (ACS), complex multivessel coronary artery disease (CAD), and increased mortality risk in ACS patients is still under investigation. Therefore, we conducted a systematic review aiming to clarify these gaps in evidence. *Methods*. We conducted a systematic search on PubMed and EMBASE with predefined keywords searching for observational studies published till August 2020. NAFLD diagnosis was accepted if confirmed through biopsy, imaging techniques, surrogate markers, or codes. Full articles that satisfied our inclusion and exclusion criteria were included in the systematic review. We used the NHLBI quality assessment tool to evaluate included studies. *Results*. Seventeen observational studies with a total study population of approximately 21 million subjects were included. Eleven studies evaluated whether NAFLD is an independent risk factor for developing ACS with conflicting results, of which eight studies demonstrated a significant association between NAFLD and ACS, mainly in Asian populations, while three reported a lack of an independent association. Conflicting results were reported in studies conducted in Europe and North America. Moreover, a total of five studies evaluated whether NAFLD and fatty liver severity in ACS patients are associated with a complex multivessel CAD disease, where all studies confirmed a significant association. Furthermore, seven out of eight studies evaluating NAFLD and hepatic steatosis severity as a predictor of all-cause and cardiovascular mortality and in-hospital major adverse cardiovascular events (MACE) in ACS patients demonstrated a significant independent association. *Conclusions*. NAFLD patients are associated with an independently increased risk of developing ACS, mainly in Asian populations, with inconsistent results in North American and European individuals. Moreover, NAFLD and hepatic steatosis severity were both independently correlated with complex multivessel CAD, mortality, and in-hospital MACE in ACS patients.

1. Introduction

Cardiovascular diseases (CVDs) account for about one-third of all deaths in the world, of which ischemic heart disease (IHD) is the greatest single cause of mortality worldwide, accounting for approximately 7 million deaths annually [1, 2]. Nonetheless, the prevalence of several metabolic disorders known to be risk factors for CVD such as nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus, dyslipidemia, and obesity has been rising dramatically lately [3, 4].

NAFLD is a multisystem complex pathology without current approved therapies, primarily affecting the liver which causes modifications to the structure and function of the liver, leading to an increased liver-related morbidity and mortality from cirrhosis, liver failure, and hepatocellular carcinoma [5–7]. Moreover, an increasing body of evidence supports that NAFLD is not only a progressive liver disease, but can also lead to multiple systemic consequences and extrahepatic manifestations, including effects exerted on the cardiovascular system (CVS) [8–11].

Interestingly, despite being a liver pathology, most deaths among NAFLD patients are due to CVD, mainly attributed to ischemic heart disease [12, 13]. Current evidence points out that NAFLD should be considered a significant independent risk factor for clinical and subclinical

CVD, increased CVD-related morbidity, and all-cause mortality [11–13]. Furthermore, the probability that NAFLD may be not only a marker but also an early mediator of atherosclerosis has been lately discussed [14]. However, several studies reported that NAFLD per se may not be causally leading to an increased cardiovascular (CV) risk [15–18].

Acute coronary syndrome (ACS) is a term that refers to any group of clinical symptoms consistent with acute myocardial ischemia. This includes unstable angina (UA), non-ST segment elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI) [19]. ACS and sudden death cause most IHD-related deaths representing 1.8 million deaths per year. The risk of acute coronary events in life is linked with the exposure to traditional cardiovascular risk factors [19]. These risk factors have also been demonstrated to also increase the susceptibility of the rapidly growing pathology, NAFLD [20–22].

Lately, several studies evaluated whether NAFLD is a predictor for an increasing risk of developing ACS, complexity of coronary artery disease (CAD), and increased mortality risk in ACS patients. However, results have been unclear with inconsistent results. Accordingly, we conducted the first systematic review to the best of our knowledge evaluating the association, complexity of CAD, all-cause and CV mortality risk, major adverse cardiovascular events (MACE), and adverse CV events of ACS in NAFLD patients through performing a systematic review.

2. Methods

This systematic review and meta-analysis was written according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [23].

2.1. Data Sources and Search Strategy. To identify potentially eligible observational studies evaluating ACS in NAFLD patients, we conducted a systematic search of PubMed and Embase from inception till the 4th of August 2020 without restrictions. The search strategy applied in these two databases included the following search string for PubMed ((“Acute Coronary Syndrome”[Mesh]) OR (“acute coronary syndrome”) OR (“Myocardial Infarction”[Mesh]) OR (“myocardial infarction”) OR (“ST Elevation Myocardial Infarction”[Mesh]) OR (“ST elevation myocardial infarction”) OR (“STEMI”) OR (“Non-ST Elevated Myocardial Infarction”[Mesh]) OR (“non-ST elevated myocardial infarction”) OR (“NSTEMI”)) AND ((“Non-alcoholic Fatty Liver Disease”[Mesh]) OR (“nonalcoholic fatty liver disease”) OR (“NAFLD”) OR (“NASH”) OR (“MAFLD”) OR (“Metabolic associated fatty liver disease”) OR (“Metabolic-dysfunction-associated fatty liver disease”)) and the following search string for Embase (‘acute coronary syndrome’/exp OR ‘acute coronary syndrome’ OR ‘myocardial infarction’/exp OR ‘myocardial infarction’ OR ‘st elevation myocardial infarction’/exp OR ‘st elevation myocardial infarction’ OR ‘stemi’ OR ‘non-st elevated myocardial infarction’/exp OR ‘non-st elevated myocardial infarction’ OR ‘nstemi’) AND (‘non-alcoholic fatty liver disease’/exp OR ‘nonalcoholic fatty liver disease’ OR ‘nafld’ OR ‘nash’ OR ‘mafld’ OR ‘metabolic associated fatty liver disease’ OR ‘metabolic-dysfunction-associated fatty liver disease’). Moreover, in order to minimize results bias, we manually searched the reference lists of pertinent articles in order to identify any additional relevant missed publications.

2.2. Study Selection and Eligibility Criteria. All observational studies evaluating the association, complexity of coronary artery disease, MACE, and mortality risk of ACS in NAFLD patients were eligible for inclusion. Original articles were included in the qualitative assessment and systematic review if they met the following inclusion criteria: (1) observational cohort population-based/hospital-based/primary care-based, case-control, descriptive studies of prospective or retrospective design; (2) hepatic steatosis confirmed based on one of the following methods: biopsy, imaging techniques such as ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), surrogate or noninvasive biomarkers of NAFLD, liver enzymes, or codes such as International Classification of Diseases (ICD); (3) confirmed diagnosis of ACS according to each study definition; (4) adult subjects (aged ≥18 years) without restrictions in terms of gender, race, or ethnicity; and (5) studies conducted on humans only.

Exclusion criteria included the following: (1) significant alcohol consumption or the presence of other secondary causes of hepatic steatosis; (2) patients with confirmed hepatitis virus of any etiology; (3) other known causes of CLD; (4) patients with confirmed cirrhosis of any etiology; (5) subjects with end-stage liver disease who are awaiting or underwent liver transplantation; (6) studies published in languages other than English, German, and Romanian; and (7) case reports, reviews, practice guidelines, commentaries, opinions, letters, editorials, short surveys, articles in press, conference abstracts, conference papers, and abstracts published without a full article.

According to the abovementioned eligibility criteria, two investigators (A.I. and S.L.P.) performed a screening evaluation independently through scrutinizing titles and abstracts excluding any apparently irrelevant studies. Subsequently, selected articles fulfilling the inclusion and exclusion criteria were further evaluated by carefully reviewing the full text. A mutual consensus was reached by discussion to resolve any discrepancies regarding study eligibility.

2.3. Data Extraction. We extracted the following information from eligible studies: author’s name, publication year, study location, study population, the source of cohort, sample size, mean age, ACS prevalence, the approach to diagnose hepatic steatosis, the number of NAFLD cases, gender, body mass index (BMI), and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, in addition to the follow-up duration and main study findings. One investigator (A.I.) extracted the data through an electronic spreadsheet, and then another investigator

(S.L.P.) reviewed the extracted data for accuracy. Discrepancies regarding the results of extracted data were settled by discussion. Extracted data was then entered into tables, while final data was collated and presented in the text of the manuscript.

2.4. Quality Assessment. Two investigators (A.I. and S.L.P.) used the National Heart, Lung, and Blood Institute (NHLBI) to independently perform the quality assessment for included studies in order to assess bias risk and internal validity in individual studies in a similar manner [24]. One tool was used for observational cohort and cross-sectional studies. The evaluation assessment tool items were answered by "yes", "no", "not applicable", "cannot determine", or "not reported". Subsequently, the studies received a rating as "good", "fair", or "poor" upon completion of the evaluation. Any discrepancies regarding quality assessment evaluation results of the two investigators were handled by discussion. Eligibility of the studies was not affected by the results of methodological quality assessment.

3. Results

3.1. Literature Search. The literature search identified 173 and 830 records from PubMed and Embase, respectively. Following the removal of 103 duplicates, we obtained a total of 900 records that were carefully reviewed through the assessment of the titles and abstracts, of which a total of 876 records were excluded due to the following reasons: (1) two hundred and ninety-seven review articles; (2) two hundred and twenty irrelevant articles; (3) two hundred and fourteen conference abstracts, papers, or reviews; (4) one hundred and four editorials, letters, notes, and short surveys; (5) seventeen articles describing CAD without clear ACS; (6) ten studies conducted on animals; (7) seven articles evaluating major adverse cardiovascular events (MACE) without clear ACS; (8) five guidelines and statements; and (9) two chapters. The eligibility of the remaining 24 articles according to the inclusion and exclusion criteria was evaluated through assessing the full text, of which seven records were excluded due to the following: (1) no clear ACS group in NAFLD patients [25, 26]; (2) opinion [27]; (3) manuscripts in Chinese and Russian languages [28, 29]; (4) article evaluating the differential expression genes of NAFLD and in acute myocardial infarction datasets [30]; and (5) an article published under hepatology elsewhere section where the full article is already included in our systematic review [31]. Hence, a total of 14 records fulfilled our inclusion and exclusion criteria and were included in our qualitative assessment and systematic review as described in Figure 1 [32–48].

3.2. Study Characteristics. The main characteristics of included studies are summarized in Table 1. A total of approximately 21 million subjects were included in this review. The number of NAFLD cases varied from 54 to 120,795, while the ACS cases varied between 80 and 16,574 with a follow-up period ranging from 6 months to 17 years in the included studies.

Six studies had a cohort study design (retrospective cohort study [35, 46], prospective cohort study [36, 37], prospective population-based cohort study [41], matched cohort study [42], nationwide population-based cohort study [43], and cohort study [44, 47]). Moreover, two studies had a cross-sectional study design (cross-sectional study [32] and cross-sectional analysis of a prospective single-center study [45]) and two observational studies (retrospective observational study [34, 40]). Furthermore, we also included a descriptive study [39] and three studies that did not clearly specify their study design [32, 38, 48].

Eight studies were conducted in Europe (Turkey $n = 4$, Italy $n = 1$, Germany $n = 1$, Finland $n = 1$, and multiple countries $n = 1$), five studies in Asia (Republic of Korea $n = 2$, China $n = 1$, Armenia $n = 1$, and Sri Lanka $n = 1$), and four studies in North America (USA $n = 2$ and Canada $n = 2$).

3.3. Quality Assessment. We used the NHLBI quality assessment tools to evaluate the methodological quality of eligible studies included in the qualitative assessment and systematic review as demonstrated in Table 2. Seven studies had an overall rating of "good" [33, 34, 36, 41–44], eight studies were rated "fair" [32, 35, 37, 39, 40, 45, 46, 48], and two studies were rated "poor" [38, 47]. Generally, all included studies clearly stated a research question or objective. The study population was specified and defined as who, where, and when in thirteen studies [33–35, 37, 39–46, 48] while six studies had a sufficient time frame [34, 41–44, 46]. Moreover, only one study evaluated hepatic steatosis more than once over the study period partially for a group of participants [34]. All but five studies assessed potential cofounding variables and adjusted statistically for their impact [37, 38, 45–47]. Furthermore, some included studies did not report a few items evaluated in the quality assessment tools.

Four out of the seven studies rated as "good" evaluating the relationship between NAFLD and ACS demonstrated a significant association between NAFLD and ACS [33, 36, 43, 44] while three studies reported a lack of an independent association [34, 41, 42]. The remaining four studies supporting this relationship were rated as "fair" [45, 46] and "poor" [38, 47]. Moreover, the association between NAFLD and complexity of CAD in ACS patients was evaluated in five studies, out of which only one was rated as "good" [33], three as "fair" [32, 45, 48], and one as "poor" [38], all supporting a more severe CAD in ACS patients with NAFLD. Furthermore, the relationship between NAFLD and adverse CV events, in-hospital MACE, all-cause mortality, and CV mortality in ACS patients was evaluated in eight studies. Two out of the three of the studies rated as "good" supported this association [36, 43] and one study opposed it [34]. The remaining five studies that supported this association were rated as "fair" [35, 37, 39, 40, 48].

3.4. Definition of NAFLD. Hepatic steatosis was evaluated using ultrasonography for diagnosing NAFLD in most studies ($n = 10$) [32, 33, 36–40, 45, 46, 48], while the others studies used codes ($n = 3$) [42, 44, 47], fatty liver index (FLI)

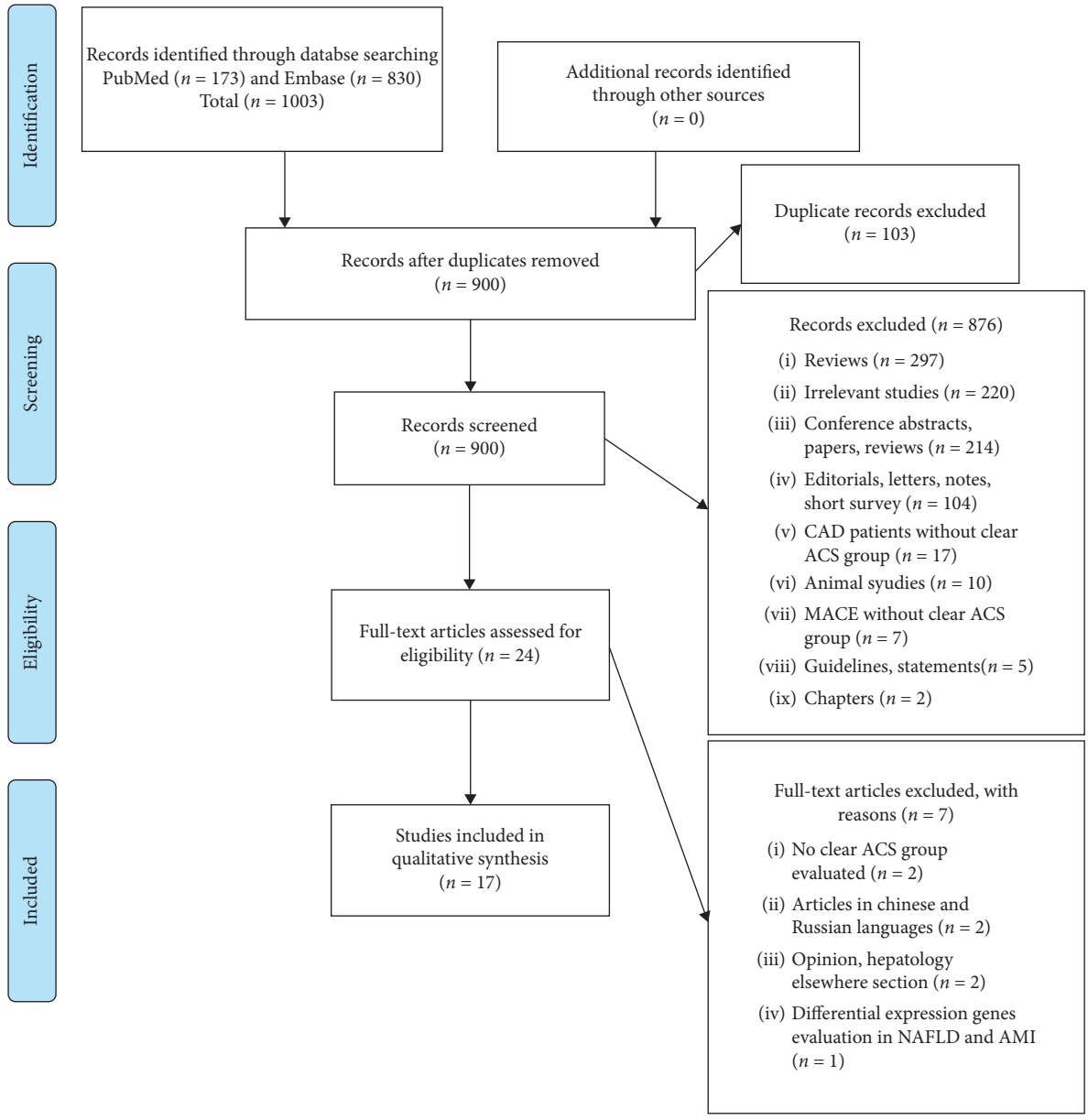

Figure 1: PRISMA flow diagram for search and selection processes of this systematic review.

($n = 2$) [41, 43], elevated ALT levels ($n = 1$) [35], and non-contrast CT imaging ($n = 1$) [34].

3.5. NAFLD as a Predictor for Developing ACS. Several studies evaluated whether NAFLD is an independent risk factor for developing ACS with conflicting results. A total of eleven studies evaluated this association, where eight studies demonstrated a significant association between NAFLD and ACS while three reported a lack of an independent association. Table 3 summarizes the current available data evaluating the association between ACS and NAFLD.

Boddi et al. evaluated 95 consecutive nondiabetic patients admitted to cardiac intensive care unit for STEMI demonstrating a very high prevalence of NAFLD evaluated using ultrasonography in the studied group [33]. A prospective cohort study conducted by Emre et al. on 186 nondiabetic patients undergoing PCI for STEMI [36]. They concluded that in-hospital nonfatal myocardial infraction (MI) was significantly greater in patients with an FLD ≥3 score ($p = 0.011$). Furthermore, Ozturk et al. compared patients with MI, stable CAD, and normal coronary arteries reporting that MI occurred predominantly in NAFLD patients evaluated using ultrasonography compared to patients with stable CAD [38]. Moreover, Kim et al. conducted a Korean nationwide population-based cohort study on 3,011,588 subjects demonstrating a HR for nonfatal MI of 2.16 (95% CI: 2.01–2.31) comparing the lowest to the highest FLI quartiles with similar results after performing a stratified analysis by age, sex, use of dyslipidemia medication, obesity, diabetes, and hypertension [43]. They concluded that FLI, a surrogate marker for NAFLD, is an independent predictor

Table 1: Studies assessing the outcomes associated with NAFLD in patients with ACS.

First author/year/country	Study design	Study characteristics	Main findings
Agac et al./2013/Turkey [32]	Cross-sectional study	(i) Total subjects: 80 (ii) Population: ACS patients (iii) ACS prevalence: STEMI: 29 (36.3%); NSTEMI: 41 (50.6%); unstable angina: 10 (12.5%) (iv) NAFLD: 65 (81.25%) (v) Mean age (years): 62.2 ± 11.2 (vi) Gender (males): 75 (78.9%) (vii) BMI: NAFLD: 28.6 ± 2.1; NAFLD absent: 25.1 ± 1.8 (viii) NAFLD diagnosis: ultrasonography (ix) AST level: — (x) ALT level: NAFLD 35 ± 17; NAFLD absent 19 ± 7 (xi) SYNTAX score: NAFLD 18 ± 8; NAFLD absent 11 ± 5 (xii) Follow up: —	NAFLD patients presented a significantly higher SYNTAX. Moreover, the stage of NAFLD correlated with SYNTAX score. In multivariate binary logistic analysis, the presence of NAFLD was an independent factor associated with supramedian SYNTAX score. In conclusion, NAFLD is a predictor of a more complex CAD in ACS patients.
Boddi et al./2013/Italy [33]	Unclear	(i) Total subjects: 95 (ii) Population: nondiabetic STEMI patients (iii) ACS prevalence: STEMI: 95 (100%) (iv) NAFLD: 83 (87.36%) (v) Mean age (years): 62.2 ± 11.2 (vi) Gender (males): 75 (78.9%) (vii) BMI: All patients: 26.0 ± 2.6; score <3 : 25.0 ± 2.5; score ≥3 : 27.2 ± 2.3 (viii) NAFLD diagnosis: ultrasonography (ix) AST level: all patients: 80 (48–183); score <3 : 76 (50–200); score ≥3 : 80 (38–183) (x) ALT level: all patients: 45 (30–68); score <3 : 32 (24–100); score ≥3 : 53 (38–68) (xi) Follow-up: —	Compared to nondiabetic STEMI patients with mild FLD, severe FLD patients were younger in age and presented a higher prevalence of multivessel CAD at logistic regression analysis; severe FLD was independently associated with a threefold risk of multivessel CAD.
Dunn et al./2013/USA [34]	Retrospective observational study	(i) Total subjects: 2,343 (ii) Population: type 2 diabetic patients (iii) ACS prevalence: MI overall: 653 (28%); <30% steatosis: 599 (28%); ≥30% steatosis: 54 (233%) (iv) NAFLD: 78 (3.33%) using ICD-9 codes; <30% steatosis: 2110; ≥30% steatosis: 233 (v) Mean age (years): <30% steatosis: 66.6 ± 15.1; ≥30% steatosis: 58.1 ± 13.7 (vi) Gender (males): 1,078 (46%) (vii) BMI: <30% steatosis: 30.8 ± 7.5; ≥30% steatosis: 36.7 ± 8.5 (viii) NAFLD diagnosis: non–contrast CT imaging (ix) AST level: <30% steatosis: 22 (17, 34); ≥30% steatosis: 26 (18, 39) (x) ALT level: — (xi) Follow-up: 5 years	Hepatic steatosis was not associated with any nonfatal adverse CV outcomes.

Table 1: Continued.

First author/year/country	Study design	Study characteristics	Main findings
Ravichandran et al./2014/Canada [35]	Retrospective cohort study	(i) Total subjects: 528 (ii) Population: ACS patients (iii) ACS prevalence: STEMI: 288 (49.3%); NSTEMI 191 (31.7%); unstable angina 76 (13%); other 29 (5%) (iv) NAFLD: 54 (10.23%) (v) Mean age (years): 63.4 (12.4) (vi) Gender (males): 402 (74.6%) (vii) BMI: — (viii) NAFLD diagnosis: elevated ALT level >90th percentile (ix) AST level: — (x) ALT level: multivariable linear regression was used to determine the change in maximum measured cardiac troponin I (cTnI) per each 1 IU/l increase in serum ALT concentration. (xi) Follow-up: 6 months	NAFLD is determined by increased ALT levels, is associated with in-hospital all-cause mortality, and up to 6 months after discharge in ACS patients.
Emre et al./2015/Turkey [36]	Prospective cohort study	(i) Total subjects: 186 (ii) Population: nondiabetic patients who underwent PCI for STEMI (iii) ACS prevalence: STEMI: 186 (100%) (iv) NAFLD: FLD score <3 : 111 (59.68%); FLD score ≥3 : 75 (40.32%) (v) Mean age (years): 58 ± 11 (vi) Gender (males): 142 (76%) (vii) BMI: all patients: 26.5 ± 2.4; score <3 : 26.0 ± 2.4; score ≥3 : 27.3 ± 2.2 (viii) NAFLD diagnosis: ultrasonography (ix) AST level: all patients: 79 ± 35; score <3 : 76 ± 35; score ≥3 : 82 ± 35 (x) ALT level: all patients: 45 ± 20; score <3 : 42 ± 19; score ≥3 : 48 ± 20 (xi) Follow-up: —	In-hospital nonfatal MI and death were significantly higher in patients with an FLD score ≥3. Using multivariate analysis, FLD score ≥3 was an independent predictor of in-hospital MACE.
Kocharyan/2016/Armenia [37]	Prospective cohort study	(i) Total subjects: 166 (ii) Population: STEMI and NSTEMI patients (iii) ACS prevalence: STEMI and NSTEMI: 166 (100%) (iv) NAFLD: 91 (54.82%) (v) Mean age (years): 63 ± 0.96 (vi) Gender (males): 116 (69.88%) (vii) BMI: — (viii) NAFLD diagnosis: ultrasonography (ix) AST level: — (x) ALT level: — (xi) Follow-up: 12 months	The presence of NAFLD in acute MI patients is associated with increased mortality.

Table 1: Continued.

First author/year/country	Study design	Study characteristics	Main findings
Ozturk et al./2016/Turkey [38]	Unclear	(i) Total subjects: 224 (ii) Population: group 1: patients with an MI-STEMI and NSTEMI; group 2: patients with stable CAD; and group 3: patients with normal coronary artery (iii) ACS prevalence: group 1: 94 (100%); STEMI: 70 (74.5%); and NSTEMI: 24 (25.5%) (iv) NAFLD: overall: 101 (45%); group 1: 66 (70.2%); group 2: 23 (38.3 %); and group 3: 12 (17.1 %) (v) Mean age (years): group 1: 60.3 ± 13.2; group 2: 57.1 ± 9.5; and group 3: 55.9 ± 7.4 (vi) Gender (males): 160 (71.43%) (vii) BMI: group 1: 25.5 ± 3.2; group 2: 25.2 ± 2.5; and group 3: 24.6 ± 3.3 (viii) NAFLD diagnosis: ultrasonography (ix) AST level: — (x) ALT level: — (xi) Gensini score: group 1: 118 ± 23; group 2: 51 ± 17; and group 3: 0 (xii) Follow-up: —	NAFLD was more prevalent in MI patients compared to stable CAD patients. Moreover, NAFLD was also significantly associated with CAD severity. Significant correlations between Gensini score and hepatic steatosis grade were reported.
Perera et al./2016/Sri Lanka [39]	Descriptive study	(i) Total subjects: 120 (ii) Population: nonfatal ACS (iii) ACS prevalence: STEMI-NAFLD: 16 (28.6); NAFLD absent: 16 (25.0); total: 32 (26.7); $p = 0.659$ NSTEMI-NAFLD: 40 (71.4); NAFLD absent: 48 (75.0); total: 88 (73.3) (iv) NAFLD: 56 (46.67%) (v) Mean age (years): 61.28 ± 11.83 (vi) Gender (males): 75 (62.5%) (vii) BMI: 24.64 ± 9.8 (viii) NAFLD diagnosis: ultrasonography (ix) AST level: — (x) ALT level: NAFLD: 62.9 ± 46.2; NAFLD absent: 29.4 ± 11.9; total: 44.9 ± 36.5 (xi) GRACE score: NAFLD: 120.2 ± 26.9; NAFLD absent: 92.3 ± 24.2; $p < 0.001$ (xii) Follow-up: 6 months	Patients with NAFLD have a higher predicted mortality from ACS during in-ward stay and at 6 months after discharge.
Keskin et al./2017/Turkey [40]	Retrospective observational study	(i) Total subjects: 360 (ii) Population: STEMI patients (iii) ACS prevalence: STEMI: 360 (100%) (iv) NAFLD: 191 (53.06%) (v) Mean age (years): 59 ± 12 (vi) Gender (males): 241 (66.94%) (vii) BMI: NAFLD absent: 27.1 ± 3.4; grade 1 NAFLD: 26.7 ± 3.4; grade 2 NAFLD: 27.0 ± 3.8; grade 3 NAFLD: 27.8 ± 3.6 (viii) NAFLD diagnosis: ultrasonography (ix) AST level: absent NAFLD: 30 ± 17; grade 1 NAFLD: 33 ± 25; grade 2 NAFLD: 33 ± 25; and grade 3 NAFLD: 36 ± 22 (x) ALT level: absent NAFLD: 24 ± 21; grade 1 NAFLD: 30 ± 24; grade 2 NAFLD: 31 ± 21; and grade 3 NAFLD: 36 ± 26 (xi) SYNTAX score: absent NAFLD: 7 ± 2; grade 1 NAFLD: 14 ± 5; grade 2 NAFLD: 20 ± 9; and grade 3 NAFLD: 26 ± 9 (xii) Follow-up: 3 years	In STEMI patients, the presence of NAFLD is correlated with unfavorable clinical outcomes, out of which, grade 3 NAFLD patients were found to have the highest mortality rates.

Table 1: Continued.

First author/year/country	Study design	Study characteristics	Main findings
Olubamwo et al./2018/Finland [41]	Prospective population-based cohort study	(i) Total subjects: 1,205 (ii) Population: STEMI patients (iii) ACS prevalence: acute MI: 269 (22.32%) (iv) NAFLD: 648 (53.78%) (v) Mean age (years): FLI <30: 51.5 (5.8); FLI 30 to <60: 52.7 (5.7); and FLI ≥60: 51.49 (5.8) (vi) Gender (males): 1,205 (100%) (vii) BMI: FLI <30: 24.3 (1.9); FLI 30 to <60: 27.3 (1.9); and FLI ≥60: 30.9 (3.3) (viii) NAFLD diagnosis: FLI (ix) AST level: — (x) ALT level: — (xi) Follow-up: 17 years	Incident CVD can be predicted using FLI. However, predicting acute MI using FLI was not demonstrated to be an independent association, mainly due to several metabolic factor interactions.
Alexander et al./2019/Italy, Netherlands, Spain, and UK [42]	Matched cohort study	(i) Total subjects: 17.7 million (ii) Population: population-based, electronic primary healthcare database (iii) ACS prevalence: Acute MI-NAFLD: 1,035; controls: 67,823 (iv) NAFLD: 120,795 (0.7%) (v) Mean age (years): Italy—NAFLD: 55.6 (14.2); controls: 54.6 (13.5); Netherlands—NAFLD: 56.1 (13.6); controls: 55.6 (13.3); Spain—NAFLD: 55.6 (13.3); controls: 54.2 (12.9); and UK—NAFLD: 53.3 (13.1); controls: 52.9 (13.2) (vi) Gender (males): Italy—NAFLD: 57.2%; controls: 54.9%; Netherlands—NAFLD: 48.6%; controls: 48.1%; Spain—NAFLD: 52.5%; controls: 48.8%; and UK—NAFLD: 51.1%; controls: 50.4% (vii) BMI: Italy—NAFLD: 29.7 (5.0); controls: 27.5 (5.0); Netherlands—NAFLD: 31.0 (5.4); controls: 28.3 (5.2); Spain—NAFLD: 31.4 (5.1); controls: 28.7 (5.1); and UK—NAFLD: 32.4 (5.9); controls: 28.5 (5.9) (viii) NAFLD diagnosis: ICD-9 codes, codes for HSD, ICPC Dutch for IPCI, ICD-19 and Read codes (ix) AST level: Italy—NAFLD: 24 (19–33); controls: 20.7 (17–25); Netherlands—NAFLD: 29 (22–40); controls: 23 (20–28); Spain—NAFLD: 29 (22–40); controls: 21 (18–27); and UK—NAFLD: 32 (24–47); controls: 22 (19–27) (x) ALT level: Italy—NAFLD: 30 (20–49); controls: 21 (16–30); Netherlands—NAFLD: 37 (25–56); controls: 25 (18–33); Spain—NAFLD: 35 (23–54); controls: 20 (15–28); and UK—NAFLD: 46 (29–69); controls: 23 (17–31) (xi) Follow-up: 2.1–5.5 years	NAFLD does not appear to be associated with acute MI risk after adjustment for established cardiovascular risk factors.

Table 1: Continued.

First author/year/country	Study design	Study characteristics	Main findings
Kim et al./2020/Republic of Korea [43]	Nationwide population-based cohort study	(i) Total subjects: 3,011,588 (ii) Population: nationwide population-based (iii) ACS prevalence: Acute MI: 16,574 (0.55%) (iv) NAFLD: According to FLI quartiles (v) Mean age (years): 51.86 ± 8.20 (Vi) Gender (males): 1,290,580 (42.9%) (vii) BMI: 23.82 ± 2.91 (viii) NAFLD diagnosis: FLI (ix) AST level: — (x) ALT level: — (xi) Follow-up: median of 6 years	FLI is an independent predictor for developing MI and CV mortality.
Labenz et al./2020/Germany [44]	Cohort study	(i) Total subjects: 44,096 (ii) Population: primary care population (iii) ACS prevalence: acute MI-NAFLD: 2.9%; controls: 2.3%; $p < 0.001$ (iv) NAFLD: 22,048 (50%) (v) Mean age (years): 55.6 (13.4) (vi) Gender (males): 50.2% (vii) BMI: — (viii) NAFLD diagnosis: ICD-10 codes (ix) AST level: — (x) ALT level: — (xi) Follow-up: 10 years	NAFLD constitutes an independent risk factor for MI in primary care in Germany.
Montemezzo et al./2020/Canada [45]	Cross-sectional analysis of a prospective single-center study	(i) Total subjects: 139 (ii) Population: ACS patients (iii) ACS prevalence: STEMI: 40 (59.7%); NSTEMI: 51 (36.6%); and UA 48 (34.3%) (iv) NAFLD: 76 (55.2%) (v) Mean age (years): overall: 59.7; CAD: 59 ± 11.62; without CAD: 54.3 ± 10.83 (vi) Gender (males): 83 (59.7%) (vii) BMI: — (viii) NAFLD diagnosis: ultrasonography (ix) AST level: with CAD: 75.6 ± 116.46; without CAD: 35.6 ± 28.42 (x) ALT level: with CAD: 55.4 ± 44.13; without CAD: 105.3 ± 147.12 (xi) Follow-up: —	NAFLD is common in ACS patients. The ultrasonographic severity of NAFLD is strongly associated with the complexity of coronary artery obstruction evaluated on angiography.
Sinn et al./2020/Korea [46]	Retrospective cohort study	(i) Total subjects: 111,492 (ii) Population: healthcare database of adults over 40 years old without history of CVD, liver disease, or cancer at baseline (iii) ACS prevalence: MI: 183 (with an overall incidence of 2.5 cases per 10,000 person-years (iii) NAFLD: 37,263 (33.42%) (iv) Mean age (years): 52.0 (8.1) (v) Gender (males): 57,123 (51.2%) (vi) BMI: 23.7 (2.9) (vii) NAFLD diagnosis: ultrasonography (viii) AST level: — (ix) ALT level: — (x) Follow-up: 725,706.9 person-years of follow-up	NAFLD was associated with a higher incidence of MI independently of established risk factors. Moreover, this finding was similar in patients in the presence and absence of more advanced NAFLD evaluated by NFS.

Table 1: Continued.

First author/year/country	Study design	Study characteristics	Main findings
Vandromme et al./2020/USA [47]	Cohort study	(i) Total subjects: 13,290 (ii) Population: hospital database of NAFLD patients using electronic signatures of disease (iii) ACS prevalence: — (iv) NAFLD: 13,290 (100%) (v) Mean age (years): 53 ± 14.7 (vi) Gender (males): 49.4% (vii) BMI: — (viii) NAFLD diagnosis: ICD-9, ICD-10, current procedural terminology, and medication mapping (ix) AST level: — (x) ALT level: — (xi) Follow-up: —	NAFLD subtype 2 was correlated with MI. When considering subtype 1 as the reference, subtype 5 was independently linked to the highest risks for MI compared to all other subtypes. Moreover, subtype 2 was also independently related to an increased risk of MI.
Xia et al./2020/China [48]	Unclear	(i) Total subjects: 325 (ii) Population: acute MI patients over the age of 60 years (iii) ACS prevalence: 100% (iv) NAFLD: 111 (34.15%) (v) Mean age (years): 70.24 ± 9.46 (vi) Gender (males): 182 (56%) (vii) BMI: — (viii) NAFLD diagnosis: ultrasonography (ix) AST level: — (x) ALT level: — (xi) Follow-up: —	NAFLD is associated with the severity of CAD, as well as being an independent predictor of adverse CV events in elderly patients with acute MI.

ACS: acute coronary syndrome; ALT: alanine aminotransferase; CAD: coronary artery disease; CT: computer tomography; CV: cardiovascular; CVD: cardiovascular disease; FLD: fatty liver disease; FLI: Fatty Liver Index; ICD: International Classification of Diseases; ICPC: International Classification of Primary Care; MI: myocardial infarction; NAFLD: nonalcoholic fatty liver disease; NFS: NAFLD Fibrosis Score; NSTEMI: non-ST-segment elevation myocardial infarction; PCI: percutaneous coronary intervention; and STEMI: ST-segment elevation myocardial infarction.

for developing acute MI. A cohort study conducted on a primary care population by Labenz et al. on 44,096 individuals demonstrated that MI patients had a significantly higher frequency of NAFLD compared to controls (2.9% vs. 2.3%, $p < 0.001$) with an obtained HR of 1.34 ($p = 0.003$) for incidence of MI in all NAFLD patients on regression analysis concluding that NAFLD is an independent risk factor for MI in primary care in Germany [44]. A cross-sectional analysis of a prospective single-center study conducted by Montemezzo et al. on 139 ACS patients concluded that NAFLD is common in ACS patients, compromising about 60% of their study population [45]. Furthermore, a retrospective cohort study conducted by Sinn et al. conducted on 111,492 individuals using a Korean healthcare database of adults over 40 years of age without any significant history of CVD, liver disease, or cancer at baseline with a total of 725,706.9 person-years of follow-up demonstrated that the cumulative incidence of MI was consistently higher in participants with NAFLD evaluated using ultrasonography compared to controls during the whole follow-up period after adjusting for established CV risk factors and medications [46]. A cohort study involving 13,290 patients with NAFLD conducted by Vandromme et al. concluded that NAFLD subtype 2 was associated with MI with an HR of 6.6 (95% CI: 3.3–13.3, $p < 0.001$) [47].

On the other hand, Dunn et al. conducted a retrospective observational study involving 2,343 type 2 diabetic patients reporting that a history of baseline myocardial infarction patients was significantly more frequent in patients with <30% hepatic steatosis evaluated using non-contrast CT imaging [34]. Moreover, a prospective population-based cohort study by Olubamwo et al. involving 1,205 STEMI patients demonstrated that incident acute MI was associated with a high FLI category with an HR of 1.65 (95% CI: 1.22–2.23) in the minimally adjusted model [41]. However, more comprehensive models including metabolic factors demonstrated a nonsignificant HR of 1.136 (95% CI: 0.777–1.662) suggesting that the predictability of acute MI using FLI might be due to several metabolic factor interactions. Furthermore, a matched cohort conducted in Netherlands, Spain, and UK by Alexander et al. involving 17.7 million individuals demonstrated a pooled HR for acute MI of 1.17 (95% CI: 1.05–1.30) after adjusting for age and smoking in NAFLD or NASH patients compared to controls [42]. Nonetheless, in a group of subjects with more details on risk factors, the HR for acute MI was 1.01 (95% CI: 0.91–1.12) after adjusting for established cardiovascular risk

Table 2: NHLBI quality assessment tool for observational cohort and cross-sectional studies.

Criteria	Agac et al. [32]	Boddi et al. [33]	Dunn et al. [34]	Ravichandran et al. [35]	Emre et al. [36]	Kocharyan [37]	Ozturk et al. [38]	Perera et al. [39]	Keskin et al. [40]	Olubunmi et al. [41]	Alexander et al. [42]	Kim et al. [43]	Labenz et al. [44]	Montemezzo et al. [45]	Sinn et al. [46]	Vandromme et al. [47]	Xia et al. [48]
(1) Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
(2) Was the study population clearly specified and defined?	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
(3) Was the participation rate of eligible persons at least 50%?	NR	Yes	Yes	NR	Yes	NR	NR	Yes	NR	Yes	Yes	Yes	Yes	NR	Yes	NR	NR
(4) Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	CD	Yes	Yes	Yes	Yes	Yes	NR	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
(5) Was a sample size justification, power description, or variance and effect estimates provided?	No	Yes	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	Yes
(6) For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	Yes	No	No

Table 2: Continued.

Criteria	Agac et al. [32]	Boddi et al. [33]	Dunn et al. [34]	Ravichandran et al. [35]	Emre et al. [36]	Kocharyan [37]	Ozturk et al. [38]	Perera et al. [39]	Keskin et al. [40]	Olubunmi et al. [41]	Alexander et al. [42]	Kim et al. [43]	Labenz et al. [44]	Montemezzo et al. [45]	Sinn et al. [46]	Vandromme et al. [47]	Xia et al. [48]
(7) Was the time frame sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	No	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	Yes	No	No
(8) For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	No	Yes	No	No	No
(9) Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
(10) Was the exposure(s) assessed more than once over time?	No	No	Partially (81 subjects)	No	No	No	No	No	No	No	No	No	No	No	No	No	No
(11) Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 2: Continued.

Criteria	Agac et al. [32]	Boddi et al. [33]	Dunn et al. [34]	Ravichandran et al. [35]	Emre et al. [36]	Kocharyan [37]	Ozturk et al. [38]	Perera et al. [39]	Keskin et al. [40]	Olubunmi et al. [41]	Alexander et al. [42]	Kim et al. [43]	Labenz et al. [44]	Montemezzo et al. [45]	Sinn et al. [46]	Vandromme et al. [47]	Xia et al. [48]
(12) Were the outcome assessors blinded to the exposure status of participants?	Yes	Yes	NR	CD	NR	NR	Yes	NR	NR	NR	NA	NA	NA	Yes	NA	NA	NR
(13) Was loss to follow-up after baseline 20% or less?	NA	NA	NA	NA	Yes	Yes	NA	NA	NA	Yes	NA	NA	NA	NA	NA	NA	NA
(14) Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
Rating	Fair	Good	Good	Fair	Good	Fair	Poor	Fair	Fair	Good	Good	Good	Good	Fair	Fair	Poor	Fair

factors concluding that NAFLD is not independently associated with acute MI.

3.6. Complexity of CAD in ACS Patients with NAFLD. A total of five studies evaluated whether the presence of NAFLD is associated with a more complex CAD disease in ACS patients, where all studies demonstrated a more severe CAD assessed using SYNTAX, GRACE, and Gensini scores and angiography in NAFLD patients.

A cross-sectional study conducted by Agac et al. involving 80 ACS patients demonstrated that NAFLD patients presented with a significantly higher SYNTAX score (18 ± 8 vs. 11 ± 5, p value $= 0.001$). Moreover, the ultrasonographic stage of NAFLD was significantly correlated with SYNTAX score by univariate analysis ($r = 0.6$, $p < 0.001$), while the presence of NAFLD was found to be an independent factor associated with supramedian SYNTAX score with an OR of 13.20 (95% CI: 2.52–69.15) concluding that NAFLD patients present with a more complex CAD [32]. Moreover, Boddi et al. demonstrated that nondiabetic STEMI patients with severe fatty liver disease were younger in age and presented with an increased prevalence of multivessel CAD compared to patients with mild NAFLD assessed by ultrasonography ($P < 0.01$), while severe fatty liver disease was independently associated with an increased threefold risk of multivessel CAD by logistic regression analysis [33]. A study conducted by Ozturk et al. involving 224 patients demonstrated that patients with MI had an increased frequency of NAFLD with stable CAD, in addition to a significant association between hepatic steatosis severity evaluated by ultrasonography with the severity of CAD assessed using Gensini score ($r = 0.648$, $p < 0.001$) [38]. A cross-sectional analysis of a prospective single-center study conducted by Montemezzo et al. concluded that NAFLD severity detected by ultrasonography is strongly related to the complexity of CAD on angiography [45]. Furthermore, Xia et al. conducted a study involving 325 acute MI patients over 60 years of age where they concluded that NAFLD is related to the severity of CAD in elderly subjects with acute MI [48].

3.7. MACE in ACS Patients with NAFLD. A total of eight studies evaluated MACE in ACS patients with NAFLD, out of which, seven reported that NAFLD is a predictor of all-cause and CV mortality and in-hospital MACE in ACS patients, while one study opposed this association.

A retrospective cohort study conducted by Ravichandran et al. involving 528 ACS patients with a follow-up period of 6 months demonstrated that NAFLD determined using elevated serum ALT is associated with an increased risk of adverse outcomes and all-cause mortality up to 6 months after discharge with an adjusted OR of 8.96 (95% CI: 3.28–24.49) in ACS patients [35]. Moreover, Emre et al. concluded that in-hospital nonfatal MI and death were both significantly increased in patients presenting a FLD ≥3 score ($p = 0.011$ and 0.041, resp.). They also conducted a multivariate analysis where an FLD ≥3 score was found to be independent predictor of in-hospital MACE with an OR of 2.454 (95% CI: 1.072–4.872, $p = 0.048$) [36]. Furthermore, Kocharyan et al. conducted a prospective cohort on 166 STEMI and NSTEMI patients with a 12-month follow-up period demonstrating that NAFLD is associated with an increased mortality ($p < 0.01$) in acute MI patients, while there was no association between the presence of NAFLD and rehospitalizations ($p > 0.05$) [37]. Perera et al. conducted a study on 120 nonfatal ACS patients concluding that NAFLD patients presented with an increased predicted mortality during in-ward stay with an adjusted OR of 31.3 (95% CI: 2.2–439.8, $p = 0.011$) and after 6 months from discharge with an adjusted OR of 15.59 (95% CI 1.6–130.6, $p = 0.011$) recommending a more aggressive treatment of CAD in NAFLD patients [39]. In addition, Keskin et al. conducted a retrospective observational study involving 360 STEMI patients reporting an in-hospital mortality rates for grade 0, 1, 2, and 3 NAFLD evaluated using ultrasonography of 4.7%, 8.3%, 11.3%, and 33.9%, respectively [40]. After a follow-up of three years, mortality rates for grade 0, 1, 2, and 3 NAFLD were 5.6%, 7.8%, 9.5%, and 33.3%, respectively. Moreover, in-hospital mortality risks were higher in grade 3 NAFLD patients using a multivariable hierarchical logistic regression analysis with an OR of 4.2 and an HR of 4.0 in a multivariable Cox proportional regression analysis. Kim et al. concluded in a nationwide population-based cohort study that FLI is an independent predictor of CV mortality with an HR of 1.98 (95% CI: 1.9–2.06). The results remained similar even after performing stratified analyses of established cardiovascular risk factors [43]. Moreover, Xia et al. reported that acute MI patients with NAFLD had a lower ejection fraction and higher rates of adverse cardiovascular event [48].

On the other hand, Dunn et al. reported that hepatic steatosis lacks the predictive value for nonfatal adverse cardiovascular outcomes in a study population involving type 2 diabetic patients [34].

4. Discussion

Recently, there is a rapidly growing interest in determining whether NAFLD and its severity are associated with ACS. To the best of our current knowledge, this is the first systematic review to evaluate the association, complexity of CAD, all-cause and CV mortality risk, in-hospital MACE, and adverse CV events of ACS in NAFLD patients. Our systematic review included 17 studies with a total study population of approximately 21 million individuals reporting results associating NAFLD with an increased independent risk for developing ACS in Asian populations. However, this independent association was inconsistent in European and North American individuals after adjusting for established CV risk factors. Moreover, we also reported a significant association relating a more advanced FLI with acute MI. Furthermore, NAFLD and hepatic steatosis severity were both significantly correlated with a more complex CAD, increased mortality, and in-hospital MACE in ACS patients. Most of these findings were demonstrated to be independently associated with NAFLD regardless of the established traditional CV risk factors across a wide range of patient populations.

Table 3: Evidence evaluating the association between ACS and NAFLD.

Condition	Country	Study population	Evidence of association	Observation
Acute myocardial infarction	USA [34]	2,343	Lack of association	Demonstrating a lack of significant association in type 2 diabetic patients only.
	Netherlands, Spain, and UK [42]	17.7 million	Weak	Significant association after adjustment for age and smoking. However, the significance was lost after adjusting for systolic blood pressure, type 2 diabetes, total cholesterol level, statin use, and hypertension.
	Turkey [38]	224	Strong	NAFLD was more frequent in MI patients.
	Korea [43]	3,011,588	Strong	FLI significantly associated with MI even after performing stratified analyses by body weight, cholesterol, age, sex, use of dyslipidemia medication, obesity, diabetes, and hypertension.
	Germany [44]	44,096	Strong	Significant association even after performing regression analysis.
	Korea [46]	111,492	Strong	Significant association even after performing adjustments for age, sex, year of visit, smoking status, alcohol intake, BMI, systolic blood pressure, fasting glucose, LDL cholesterol, use of antihypertensive medications, use of antidiabetic medications, use of lipid-lowering medications, and use of aspirin and antithrombotic medications at baseline.
	USA [47]	13,290	Strong	NAFLD subtypes 2 and 5 were independently significantly associated with MI.
STEMI	Finland [41]	1,205	Weak	FLI is associated with MI in minimally adjusted models. However, it lost significance in most comprehensive models with metabolic factors.
	Italy [33]	95	Strong	High prevalence of NAFLD in nondiabetic patients admitted for STEMI.
	Turkey [36]	186	Strong	Severe FLD is an independent predictor of STEMI by performing multivariate analysis.
ACS	Canada [45]	139	Strong	60.5% of severe CAD patients had NAFLD.

ACS: acute coronary syndrome; FLD: fatty liver disease; FLI: Fatty Liver Index; LDL: low-density lipoproteins; MI: myocardial infarction; NAFLD: nonalcoholic fatty liver disease; and STEMI: ST-segment elevation myocardial infarction.

In our systematic review, we reported several findings that need to be further discussed. Firstly, in order to reflect our current knowledge about NAFLD, this term was recently updated to metabolic-dysfunction-associated fatty liver disease (MAFLD) with newly defined diagnostic criteria [49, 50]. However, these two terms, NAFLD and MAFLD, should not be used interchangeably due to the existing differences between them. All studies evaluated in the current systematic review used the diagnostic criteria of NAFLD and not MAFLD; therefore, our findings reflect the association in NAFLD and not MAFLD. Interestingly, MAFLD definition was demonstrated to be more practical for identifying fatty liver disease (FLD) patients with an increased risk of disease progression [51].

Secondly, we observed a variety of methods that were used to detect hepatic steatosis and diagnose NAFLD. A positive diagnosis of NAFLD can be confirmed through confirming the presence of hepatic steatosis by histology which is the current gold standard, as well as imaging methods such as ultrasonography which is the most common imagistic assessment used, CT scans and MRI, in addition to noninvasive assessment through surrogate markers [20, 52]. Most studies included in our systematic review used ultrasonography for diagnosing NAFLD. Despite demonstrating a low sensitivity when hepatic steatosis is less than 20% on biopsy, ultrasonography remains the preferred initial first-line imaging method for assessing liver fat with a sensitivity and specificity of 84.8% and 93.6%, respectively [53, 54]. Moreover, a couple of studies used surrogate markers to evaluate hepatic steatosis including FLI and ALT levels. The FLI was demonstrated to be a simple and accurate predictor of hepatic steatosis in the general population [55]. On the other hand, evidence demonstrated that solo use of liver enzymes such as ALT levels is a poor predictor of NAFLD as approximately 70–80% of patients may have normal range levels and therefore is not helpful for diagnosing or evaluating the severity of the disease [56, 57].

Thirdly, we noticed that most included studies supported the presence of an independent association linking NAFLD with an increased risk of ACS. However, three studies opposed this association, out of which one study was a matched cohort study involving 17.7 million European individuals demonstrating the presence of this association which lost its significance after adjusting for established CV risk factors in a group of subjects with more complete data on risk factors. Although studies conducted on European and American populations reported inconsistent results, interestingly, all studies conducted on Asian populations reported a significant independent association between NAFLD and an increased risk of ACS. This might be explained by the different

lifestyles and epidemiological characteristics as well as eating habits compared with Western subjects. Therefore, taking into consideration the different populations with distinct key contributing characteristics should not be neglected while elaborating the current results. Another explanation that might be attributing to these inconsistent results can be explained by the common mutual CV risk factors such as obesity, diabetes, dyslipidemia, genes, and other parameters that are present in both diseases.

Fourthly, the complexity of CAD in ACS was assessed using several different methods including thorough angiography, in addition to the SYNTAX, GRACE, and Gensini scores. All these methods have been demonstrated to be useful in evaluating the severity and extent of atherosclerosis in CAD patients presenting with ACS [58, 59].

Fifthly, an independent relationship linking increased in-hospital MACE and all-cause and CV mortality in ACS patients with NAFLD and hepatic steatosis severity was reported in most studies. However, only one study opposed this association which was conducted on type 2 diabetic patients [34]. Therefore, the results obtained in this study cannot be generalized on the general population.

Sixthly, the quality assessment of studies included in our systematic review demonstrated that the majority of studies that are currently published in the literature evaluating the association of interest are of "fair" quality making up eight studies out of seventeen, followed by seven studies that were rated as "good" and only two studies rated as "fair". Therefore, results obtained by studies with "fair" and "poor" ratings should be interpreted with caution because of the increased risk of bias and possible methodological flaws.

Our systematic review has several limitations which should be mentioned. First, the observational design of the studies included in this review does not allow us to establish a clear causal correlation between NAFLD and ACS, complexity of CAD, or mortalities. Second, most included studies assessed hepatic steatosis using ultrasonography and to a lesser extent FLI, ALT levels, and CT, whereas none of the studies used liver biopsy which is the current gold standard for diagnosing and staging of NAFLD. This can possibly under- or overestimate the prevalence of NAFLD. However, we did not exclude studies using surrogate markers or liver enzymes as we wanted our study to be thorough and comprehensive by covering all studies published till the search date evaluating the studied associations. Hence, we can have more generalizable results with more significance. Third, despite having two included studies of "poor" quality, most included studies were rated as either "fair" or "good", therefore associating the results with a lower risk of bias.

Nevertheless, our systematic review also presents several important strengths. The topic of this systematic review is of important clinical relevance due to the rapid increase of prevalence in NAFLD worldwide, in addition to the higher related morbidity and mortality associated with ACS. We believe that the current review outlines and summarizes the current literature. It also points out the missing required data to be evaluated in further future studies. Moreover, this systematic review was conducted comprehensively, therefore, covering the current published studies evaluating the studied associations in a systematic manner. To the best of our knowledge, this is the first systematic review to evaluate the association, complexity of CAD, and all-cause and CV mortality in ACS patients with NAFLD.

5. Conclusions and Future Directions

In conclusion, NAFLD patients are associated with an independently increased risk of developing ACS, mainly in Asian populations. However, this association was inconsistent in studies conducted on individuals from North American and European backgrounds. Moreover, NAFLD and hepatic steatosis severity were both demonstrated to be independently correlated with complex multivessel CAD, all-cause and CV mortality, in addition to in-hospital MACE in ACS patients.

Therefore, due to the higher predicted MACE and mortality rates in ACS patients with FLD, we recommend screening for hepatic steatosis using the newly defined MAFLD diagnostic criteria in order to identify FLD patients with an increased risk for disease progression, also requiring a thorough CV risk assessment. Early monitoring and identification of patients with MAFLD will allow enhancing the management plans and modifying the underlying risk factors, reducing the overall incidence of adverse events and improving the overall prognosis as well as promoting survival. Furthermore, FLD patients from different racial backgrounds should be evaluated accordingly while stratifying for CV risk, especially in ACS, due to the different contributing distinct characteristics that should not be neglected.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

D. D. had the idea of the manuscript. A. I. and S. L. P. independently applied the search strategy and performed the study selection, data extraction, and risk-of-bias assessment. A. I. drafted the manuscript. D. D. and S. L. P. contributed to the writing of the manuscript. D. D. made substantial contributions to the conception and critically revised the manuscript for important intellectual content. All authors revised the final manuscript and approved the final version.

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Review Article

Nonalcoholic Fatty Liver Disease and Sarcopenia: Where Do We Stand?

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The link between metabolic syndrome (MetS) and sarcopenia has not been extensively studied, but it is evident that they share several common features. Crucial mechanisms involved in sarcopenia-nonalcoholic fatty liver disease (NAFLD) interplay are based on effects of insulin resistance, chronic inflammation, oxidative stress, and crosstalk between organs by secretion of cytokines (hepatokines, adipokines, and myokines). Currently, published studies confirm the association of sarcopenia with the degree of NAFLD defined by liver histology. However, prospective studies that will give us information regarding the causal effect of NAFLD and sarcopenia are still needed. Furthermore, there is a need for a patient-friendly, noninvasive, low-cost method for detection of loss of skeletal muscle mass, strength, and physical performance in the context of NAFLD. Moreover, potential treatment strategies such as physical exercise and nutritional supplementation, that are usually a part of management of sarcopenia, should also be investigated in NAFLD patients, especially given the fact that for now, we do not have a good treatment option for NAFLD. Therefore, future investigations should combine studies on NAFLD and sarcopenia in terms of physical activity and nutritional interventions such as vitamin D supplementation. This review aims to report recent evidence concerning the links between sarcopenia and NAFLD and methods to assess sarcopenia.

1. Introduction

During the last few decades, we have witnessed the number of changes due to aging of population, and several growing aging-related health problems need to be addressed by geriatric researchers, including sarcopenia. Sarcopenia was first described at the end of 20th century, and the term is coined using two Greek words: sarx (flesh) and penia (loss) [1]. The first official definition of sarcopenia was given by the European Working Group on Sarcopenia in Older People (EGWGSOP) as a loss of skeletal muscle mass accompanied with low muscle strength and decreased physical performance [2]. More recent guidelines (EWGSOP2) suggest that the first diagnostic criterion for sarcopenia is low muscle strength, which can be easily measured with dynamometry. If the low muscle strength is detected, low muscle quantity or quality confirms sarcopenia [3]. Nowadays, sarcopenia is often considered to be a comorbid disease. Primary

sarcopenia is associated with aging (loss of muscle mass and strength), while a secondary sarcopenia develops because of underlying diseases, lack of physical activity, or inadequate nutrition [4]. Sarcopenic patients are at greater risk of a metabolic impairment, prolonged hospital stay, delayed healing, falls, wound infections, and poor surgery outcomes [5].

Prevalence of sarcopenia varies from 6% to 24% (age and gender adjusted), depending on the criteria used to determine muscle mass and strength. The prevalence increases with age, and it can reach >50% after the age of 80 [6]. To some extent, sarcopenia is a physiologic process that starts between the ages of 30 and 40, and it aggravates after the age of 60 when every year 3% of muscle strength is lost [7]. There is no standard or universally efficient therapy for sarcopenia; so, the most important strategies are physical therapy and/or resistance training together with nutrition support. High-protein diet enriched with special anabolic pharmaconutrients (such as b-hydroxy-b-methylbutyrate and leucine) and vitamin D supplementation should be encouraged [8].

Sarcopenia is well defined in the elderly, but it is also often encountered in patients of all ages with acute and chronic muscle-wasting diseases, such as cancer, chronic heart failure, chronic obstructive pulmonary disease, neuromuscular diseases, chronic kidney disease, liver diseases, autoimmune and inflammatory diseases, chronic infection, and polymorbidity [9]. The skeletal muscle is the primary organ of insulin-mediated glucose disposal. Additionally, decreased muscle mass has a crucial role in insulin resistance (IR) and metabolic syndrome (MetS). Thus, it is not surprising that, recently, it was found that sarcopenia is frequently associated with cardiometabolic disorders including MetS, diabetes mellitus (DM), and cardiovascular disease [10]. Also, there is a growing interest in the involvement of skeletal muscle mass in chronic liver disease (CLD), namely, liver cirrhosis, end-stage liver disease (ESLD), and nonalcoholic fatty liver disease (NAFLD) [11].

Sarcopenic obesity is characterized by decreased lean body mass accompanied with excessive adipose tissue accumulation. Obesity aggravates sarcopenia, impairs physical performance, and increases mortality rates [12]. Adipose tissue releases adipokines that regulate lipid metabolism, impact insulin sensitivity, liver fatty infiltration, and fibrogenesis. Also, sarcopenia and sarcopenic obesity are recognized as independent risk factors for the development of NAFLD and liver fibrosis [13].

The link between MetS and sarcopenia has not been extensively studied, but it is evident that there are several common features of both phenomena. Obesity and IR are considered to play the central role in both MetS and sarcopenia [7, 9]. Since NAFLD is regarded as the liver manifestation of MetS, there is an interplay between these two diseases. Patients with MetS are often presenting with loss of muscle mass and the accumulation of intramuscular fat as a result of the complex interplay of inadequate nutritional intake and physical inactivity, insulin resistance, oxidative stress, proinflammatory cytokines, hormonal changes, and mitochondrial dysfunction [7, 9].

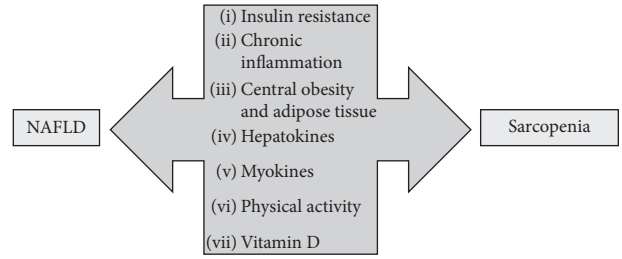

Figure 1: Possible mechanisms of the interaction between NAFLD and sarcopenia. ∗NAFLD, nonalcoholic fatty liver disease.

Glucose is disposed primarily in the skeletal muscle in an insulin-responsive manner, and the loss of muscle mass may lead to insulin resistance. Furthermore, chronic low-grade inflammation inherent in obesity and central obesity, vitamin D deficiency, physical inactivity, hepatokines, and myokines might play a role in the mechanistic background of sarcopenia and NAFLD [14]. Loss of muscle mass and function induce contractile impairment and plethora of metabolic and endocrine disruptions. Therefore, sarcopenia can affect whole-body metabolism and the immune and inflammatory responses [7]. Sarcopenia could be considered as one of the causative factors for development of NAFLD and should be assessed and tackled as a part of the broad assessment and therapeutic approach to the disease [11]. This review aims to report recent evidence concerning the links between sarcopenia and NAFLD and methods to assess sarcopenia.

2. Mechanisms of Interplay between NAFLD and Sarcopenia

According to recent data, sarcopenia is a common complication of liver cirrhosis and is observed in more than half of patients with ESLD [15]. Also, sarcopenic obesity is a common finding in patients with cirrhosis and obesity. Sarcopenia in liver cirrhosis is associated with increased mortality, hyperammonemia and overt hepatic encephalopathy, increased incidence of infections and sepsis, and an increased length of hospital stay after liver transplantation (LT) [16–20]. Moreover, Berzigotti et al., in their two studies, have showed that obesity defined by increased BMI is an important predictor of decompensation of liver cirrhosis in patients with compensated cirrhosis of various etiologies. This effect was independent of some cofounders such as albumin and portal hypertension. According to these studies, liver cirrhosis decompensation occurred in 14% of patients with normal weight, in 31% of overweight, and in 43% of patients with obesity [21, 22]. As it was mentioned, sarcopenia is recognized as one of the risk factors of NAFLD that is the most common cause of CLD and the rapidly rising indication for LT. NAFLD is closely related to MetS, and its individual component, but the main factor, involved in NAFLD pathogenesis is IR [15, 16]. NAFLD is a syndrome that includes a wide spectrum of histopathological alterations ranging from nonalcoholic fatty liver (NAFL) or

simple steatosis to nonalcoholic steatohepatitis (NASH) and fibrosis, and finally, cirrhosis and hepatocellular carcinoma (HCC) [15–17]. In recent years, the complex relationship between sarcopenia and NAFLD/NASH has been a focus of research interest [18]. Thus, considerable body of evidence has emerged on the significant interplay between pathophysiological mechanisms of NAFLD and sarcopenia. Given the fact that many of them are shared, it is challenging to decide whether sarcopenia is the cause or the consequence of NAFLD. Crucial mechanisms involved in sarcopenia-NAFLD interplay are based on effects of IR, chronic inflammation, oxidative stress, and crosstalk between organs by secretion of cytokines (hepatokines, adipokines, and myokines) [17–20] (Figure 1).

2.1. Insulin Resistance. It is well known that insulin plays a crucial role in glucose metabolism, and that the liver and the skeletal muscle are target organs of insulin. Both the liver and muscle glycogen contribute to the homeostasis of the energy metabolism in the human body. IR is a pathological condition in which cells fail to respond normally to the insulin [17]. IR is a consequence of fat tissue infiltration in the skeletal muscle accompanied by increased circulating free-fatty acid (FFA) from excessive body fat [14]. Furthermore, IR of the skeletal muscle leads to reduction of protein synthesis and increased muscle degradation, which contributes to muscle mass loss. Thus, IR has a pivotal role in sarcopenia development. On the other hand, reduced muscle mass promotes IR [14, 17]. Skeletal muscles by the expression of the insulin-dependent transporter GLUT-4 have a primary role for whole-body glucose homeostasis. In the case of decreased insulin sensitivity, the uptake of glucose is impaired, and insulin stimulated glycogen synthesis [14]. Consequently, there is an increased conversion of glucose to the triacylglycerol in the liver, which leads to development of the fatty liver. This process is responsible for hepatic IR. Moreover, obesity promotes an increased flux of FFA [14]. Fatty liver infiltration is connected more to skeletal IR than liver IR in NAFLD patients, and that observation supports the hypothesis that skeletal muscle IR has the pivotal role in NAFLD development [14, 23]. Therefore, IR is the most important pathophysiological mechanism involved in development of sarcopenia and NAFLD. On the other hand, sarcopenia promotes IR, independent of obesity, because the skeletal muscle is the primary tissue responsible for insulin-mediated glucose disposal. Furthermore, myosteatosis also promotes IR; thus, the presence of both sarcopenia and obesity are acting together in promoting IR and dysglycemia. The presence of both components, liver injury and sarcopenia, independently or in combination with other confounders, such as obesity, aging, and diabetes mellitus type 2 (T2DM), acts synergistically leading to progression of IR and dysglycemia. IR induces disturbances in function of skeletal muscles, the liver, and adipose tissue [15, 24, 25].

2.2. Adipose Tissue. Obesity is a global health problem and an increasing global burden of metabolic, cardiovascular, and malignant morbidity and mortality. A reciprocal interaction among sarcopenia and excess visceral fat aggravates loss of muscle mass [14]. Adipose tissue is the third player in the field of interaction between NAFLD and sarcopenic muscle. Its effects are most pronounced in obesity. The coexistence of sarcopenia and obesity is defined as sarcopenic obesity and recognized as a chronic inflammatory state. Adipose tissue and skeletal muscle inflammation synergistically lead to liver injury and aggravation of sarcopenia. In obesity, adipose tissue inflammation leads to increased secretion of proinflammatory cytokines (e.g., tumor necrosis factor alfa (TNF-α) and interleukin 6 (IL-6)) and adipokines (e.g., leptin, adiponectin, resistin, and irisin). TNF-α interferes with insulin receptor activity, whereas IL-6 blocks insulin signaling and glucose uptake leading to deterioration of IR. Adding to the complexity of liver-skeletal muscle-adipose tissue axis, it seems that IL-6 and irisin have both proinflammatory effects when acting as adipokines and anti-inflammatory effects as myokine substances [23, 26–30].

Proinflammatory cytokines such as TNF-α and IL-6 decrease the adiponectin level. Additionally, myostatin may simultaneously increase adipose tissue mass and decrease the level of adiponectin secretion in adipose tissue. Low level of adiponectin is related with decreased insulin signaling and fatty acid β-oxidation in the liver and muscles cells, encouraging an important pathophysiological mechanism in NAFLD and sarcopenia [23, 26–30]. The interplay between adiponectin and myostatin actions within the muscle, liver, and adipose tissue is complex, supporting the vicious circle of perpetuation of all involved mechanisms in damage of target organs.

Increased adipokine leptin secretion in inflammed adipose tissue is associated with decreased energy expenditure, dyslipidaemia, obesity, and IR. In addition, leptin promotes secretion of TNF-α and IL-6, strengthening the impact of the inflammatory process in adipose tissue. In NAFLD, it contributes to steatosis and fibrosis, whereas in skeletal muscles, it acts as an anabolic substance. In addition to adipose tissue, leptin could be secreted by skeletal muscles too. Interestingly, when secreted as myokine, leptin acts as a liver protective substance. Unfortunately, the state of sarcopenia limits its autocrine anabolic effects along with remote protective effects on the liver [23, 26–30].

2.3. Chronic Low-Grade Inflammation. Inflammation and oxidative stress are shared, and mutually perpetuating pathogenetic mechanisms are involved in IR, sarcopenia, and NAFLD. In addition, obesity, often coexistant with NAFLD, is also recognized as a chronic inflammatory state characterized by increased levels of cytokines and infiltration of adipose tissue with proinflammatory cell types, most notably macrophages. TNF-α acts by stimulating reactive oxygen species production and causes oxidative stress and mitochondrial dysfunction. Additionally, it also inactivates the AMP-activated protein kinase pathway, which relates to

NAFLD development. Proinflammatory cytokine IL-6 also plays an important role in systemic inflammation and NAFLD/NASH development. Both cytokines have a negative association with the skeletal muscle. Many studies have confirmed the association of high systemic levels of cytokines (e.g., TNF-α, high-sensitivity *C*-reactive protein (hs-CRP), and IL-6) with low muscle mass and progressive course of NAFLD [18, 24, 28, 31]. For example, Hong et al. [32] showed in their study that patients who had sarcopenia also had higher levels of hs-CRP in comparison to the patients without sarcopenia. Interestingly, authors had showed that hs-CRP levels had a significant negative correlation with skeletal muscle mass index and liver attenuation index. These data are suggesting that inflammation can be involved in the pathogenesis of sarcopenia and NAFLD [32].

2.4. Liver and Hepatokines. Excessive FFA oxidation in NAFLD promotes formation of oxygen free radicals, which cause lipid peroxidation and production of proinflammatory cytokines (e.g., TNF-α, transforming growth factor-β (TGF-β)). Except direct liver injury and subsequent development and progression of liver fibrosis, several hepatokines (e.g., fetuin *A* and *B*, selenoprotein *P*, fibroblast growth factor 21 (FGF12), leukocyte cell-derived chemotaxin 2 (LECT2), and hepassocin (HPS)) are produced. By its auto-, para-, and endocrine function, these affect IR, protein catabolism, lipid metabolism, and sarcopenia, explaining the possible link between the liver, adipose, and muscle tissues [27, 33].

2.5. Skeletal Muscles and Myokines. Skeletal muscles account for about 40–50% of lean body mass. Given the fact that it is the most important tissue responsible for insulin-mediated postprandial glucose disposal, skeletal muscles act as a pivotal factor in glucose and energy homeostasis. Loss of muscle mass leads to the metabolic disturbances, decreased insulin action and signaling-IR, reduced gluconeogenesis, glucose intolerance, pronounced production of triacylglycerol, and exacerbation of proteolysis, which eventually lead to the vicious circle of further aggravation of IR, severity of NAFLD, and muscle consumption [34–36]. Therefore, IR is characterized by disruption of protein metabolism because the mammalian target of the rapamycin pathway remains inactive and cannot inhibit autophagy or lysosomal degradation of proteins and organelles involved in muscle catabolism.

Skeletal muscles secrete myokines and peptides involved in pathophysiological mechanisms of NAFLD. Among them, exercise-induced secretion of IL-6 and irisin has a protective role against NAFLD development in obese patients [37, 38]. Irisin plays a critical role in muscle energy metabolism by increasing energy expenditure due to heat loss and the liver by fatty acid β-oxidation [39]. IL-6 within skeletal muscle promotes myogenic differentiation, basal and insulin-stimulated glucose uptake, fatty acid β-oxidation, and lipolysis. In the liver, IL-6 acts anti-inflammatory by increasing glucose production and fatty acid β-oxidation. Unfortunately, with muscle loss, decreased secretion of both protective myokines can be expected. In inflammation and physical inactivity, skeletal muscles produce a TGF-β superfamily member—myostatin. Its autocrine actions inhibit muscle growth and differentiation by activation of proteolytic pathways and inhibition of protein synthesis and regeneration. Myostatin receptors are also present on hepatic stellate cells, inaugurating the link between muscle and liver tissues. It is still unknown whether fatty liver promotes sarcopenia by activation of myostatin production in skeletal muscles or whether sarcopenia promotes liver disease by myostatin-related activation of hepatic stellate cells [15, 18, 40]. Furthermore, we know that obesity is associated with low levels of adiponectin. Myostatin also increase adipose tissue mass, which is connected to the decreased adiponectin secretion [15].

All these explain the direct (independent of insulin effects on adipose tissue) relationship between NAFLD and sarcopenia.

2.6. Physical Activity. Physical inactivity decreases muscle mass and interferes with the production profile of myokines and their effects on prevention of further muscle loss and accumulation of intrahepatic fat [27]. Myokine irisin secretion is induced by exercise, possibly explaining negative effects of physical inactivity on liver steatosis. There is also a link between physical activity and the production of hepatokines (e.g., exercise promoted decrease in secretion of hepatic and muscle IR promoter fetuin *A* and increase in secretion of myostatin inhibitor follistatin) [27]. Furthermore, loss of muscle strength and continuation of physical inactivity is a risk factor for more progressive muscle loss, fat accumulation, and aggravation of inflammation, leading to the vicious cycle of repetitive physical inactivity and even more pronounced sarcopenia [27].

2.7. Vitamin D. Vitamin D receptor is expressed in various cells including the liver and skeletal muscles. In addition to pancreatic beta cells, vitamin D regulates expression of the insulin receptors in peripheral target tissues too. It is a potent arbitrator in development of IR, MetS, NAFLD, and sarcopenia. In muscles, it plays an important role in myoblast proliferation and differentiation, skeletal muscle growth, and as an attenuator of muscle inflammation. In the NAFLD liver, vitamin D deficiency likely contributes to disease worsening by promotion of inflammation-mediated pathways and amplification of liver fibrosis [41, 42].

3. Diagnosis and Assessment of Sarcopenia in Patients with NAFLD

In both the literature and everyday clinical practice, we can find different tools and criteria to measure muscle mass and define sarcopenia. Traditionally, the term sarcopenia has been used to define loss of muscle mass in the aging population [1]. The European Working Group on Sarcopenia in Older People (EWGSOP) and the Asian Working Group for Sarcopenia (AWGS) recommend using the presence of both low muscle mass and low muscle function (strength or performance) for the diagnosis of sarcopenia [2, 3], thus

acknowledging the importance of muscle quality and quantity for clinical outcomes. EWGSOP2, the updated consensus paper on sarcopenia, focuses on low muscle strength as a key characteristic of sarcopenia, given that the negative clinical outcomes are limited to patients with impaired muscle strength and/or function [3]. In a recent study, handgrip strength combined with the model for end-stage liver disease (MELD) score was shown to be the superior predictive model among commonly employed techniques to diagnose sarcopenia in cirrhosis [43].

On the other hand, the North American Working Group on Sarcopenia in Liver Transplantation defines sarcopenia using only muscle mass assessed by CT scan at the L3 level based on assumption that skeletal muscle depletion is the most clinically relevant parameter, least susceptible to various influences that can be objectively measured in clinical practice [44]. It has been shown that muscle mass does not always correlate well with muscle strength or function in the cirrhotic population [45, 46]. Furthermore, when compared with the other modalities, CT scan alone can identify the highest percentage of muscle loss in cirrhosis, which poses a significant risk of over diagnosing sarcopenia in patients without cirrhosis [47, 48]. Unresolved issues in diagnosis of sarcopenia in liver disease with no standardized protocols and clear cutoff points have implications for the accuracy and reproducibility of studies in the field and limit its widespread application in the clinical practice.

3.1. Assessment of Muscle Mass. One of the issues in defining sarcopenia lies in different skeletal muscle mass indices that have been suggested for its assessment. When evaluating the adequacy of muscle mass, the absolute level of skeletal muscle mass has been used after adjusting for body size using height squared (SM/ht^2), weight (SM/wt), or body mass index (SM/BMI). SM/ht^2 was first suggested by Baumgartner et al. in the New Mexico Elder Health Survey [49]. Defined in this way, sarcopenia was significantly associated with physical disability, but subjects with a greater BMI are less likely to be classified as having sarcopenia [50]. Janssen and coworkers proposed weight-adjusted muscle mass index (SM/wt), which is suggested to be the more appropriate index for obese patients [51]. More recently, Foundation for the National Institutes of Health Sarcopenia Project in 2014 recommended adjusting the appendicular lean mass using body mass index (ASM/BMI) to obtain the parameter that is most strongly and directly correlated with weakness and slowness [52]. Present, there is no gold standard for the assessment of muscle mass in patients with NAFLD.

Although traditional anthropometric measures cannot differentiate fat from muscle, some methods, such as midarm muscle circumference (MAMC), midarm muscular area (MAMA = (MAMC)^2/4 × 0.314), and triceps skinfold (TSF), are still used in clinical practice because they are safe, readily available, inexpensive, and relatively not affected by fluid retention. In trained hands, these measurements have good intra- and interobserver agreement (intraclass correlation of 0.8 and 0.9 for TSF and MAMC, respectively) [53]. Both MAMC and TSF have demonstrated a good prognostic value for mortality among patients with cirrhosis [54], and low MAMC was found to be an independent predictor of mortality after liver transplant [47] and in a large sample of the general male population [55]. MAMC below the 10th percentile of an age- and sex-matched population is considered for the diagnosis of sarcopenia [47].

It has been shown that ultrasound measures of muscle depths can be used to predict overall skeletal muscle mass, and that appendicular lean body mass data reliably correlate with those derived from DXA scores in older adults [56]. The EuGMS sarcopenia group recently proposed a consensus protocol for using ultrasound in muscle assessment, including measurement of muscle thickness, cross-sectional area, fascicle length, pennation angle, and echogenicity [57]. Japanese authors described a method of estimating the cross-sectional area of the psoas muscle in a healthy population [58]. In patients with cirrhosis, iliopsoas muscle index (IP index, iliopsoas muscle area/height^2) derived by ultrasound showed a good correlation with CT-based measurements of the muscle loss [59]. In the European population, psoas to height ratio was significantly associated with mortality in a cohort of 75 patients with decompensated cirrhosis [60]. In a recent study, authors proposed a model for the evaluation of sarcopenia using ultrasonic measurement of the thigh muscle thickness and body mass index, which is moderately accurate in comparison to psoas CT/MR measurements, with a receiver operating characteristic area under the curve of 0.78 in men and 0.89 in women [61]. Although ultrasound is an important addition to the diagnostic toolbox of sarcopenia with its noninvasive, easy, portable approach, with reliable and valid data available for older adults, more research is needed to validate prediction equations for those with varying health conditions, including chronic liver disease [62].

Bioimpedance analysis (BIA) is a commonly used method for body composition assessments in both clinical practice and research settings. It is a noninvasive, relatively cheap, and simple technique that can measure the volume of fat and lean body mass by estimating total body water. So, body composition assessment from BIA relies on a calibration equation developed using a reference method such as DXA, CT, or MR. For this reason, it is important to standardize the cutoff values for diagnostic purposes in each population. BIA prediction equation to estimate total body skeletal muscle mass (SM) was generated from the study of Janssen and coworkers who validated BIA against SM obtained from MRI. [63, 64] and adjusted muscle mass by weight (SMI = SM/wt, %). Low SMI was defined as a SMI below one standard deviation of young adult values according to the data from the Third National Health and Nutrition Examination Survey (NHANES III) [63]. In a subsequent study, the same group presented skeletal muscle cutpoints for physical disability risk in older adults where in which skeletal muscle was normalized for height. Severe sarcopenia is defined when SMI is ≤8.5 kg/m^2 (men) or ≤5.75 kg/m^2 (women) [63, 64]. These cutoff values are used in the EWGSOP consensus when absolute SM is estimated from BIA [12]. As it was mentioned earlier, data from the

NHANES III population study showed that severe hepatic steatosis was associated with a decreased risk of sarcopenia as defined by the height-adjusted SMI (odds ratio (OR) 0.63; 95% confidence interval (CI) 0.46–0.87), but at the same time, it was associated with an increased risk of sarcopenia as defined by the weight-adjusted SMI (OR 1.73; 95% CI 1.31–2.28) [65]. These observations suggest that the definition of sarcopenia may explain the conflicting results regarding the relationship between sarcopenia and NAFLD. In a study from Japanese NAFLD population, there was a higher prevalence of reduced muscle mass using sarcopenia index (ASM/BMI) and the skeletal muscle mass/fat mass ratio (SF) compared to the high adjusted appendicular skeletal mass (SMI). Unlike SMI, sarcopenic index and SF ratio correlated with increasing severity of NAFLD (defined by fibrosis stage and NAFLD activity score (NAS)) [66]. There is a significant impact of adiposity on the validity of BIA and other 2 compartment methods for the assessment of fat free mass. However, the overestimation of fat free mass in obesity can be improved by using a correction factor for subjects with BMI $\geq 30\,kg/m^2$ [67].

Dual energy X-ray absorptiometry (DEXA) body composition uses low-dose X-rays to provide a whole-body or regional scan and analyze fat, bone mineral, and lean tissues. The method is precise and reproducible (coefficient of variation 0.5%) [68], but cost and access are an issue in many parts of the world [45]. According to the EWGSOP, it represents the preferred alternative method to CT and MRI in the research setting and clinical practice [3]. DXA specific measures of LM include lean mass index (LMI: total LM/height^2), appendicular lean mass (ALM: arms LM + legs LM), and appendicular lean mass index adjusted for BMI and height (ALMI: ALM/BMI, ALM/height^2), and the current EWGSOP recommendations focus on cutoff points usually set at −2 standard deviations compared to the mean reference value (healthy young adults) [3]. DXA-derived LM is higher than skeletal muscle mass measured by CT or MRI because it includes the sum of body water, total body protein, carbohydrates, nonfat lipids, and soft tissue mineral [69]. Although there are conflicting reports on the influence of excess body water on DEXA measurements, the use of ALM has been proposed to minimize confounding by ascites in patients with cirrhosis [47]. To avoid possible further overestimation of LM by lower limb edema, a group from Australia proposed a measurement of upper limb LM, which was most strongly associated with waitlist mortality as compared to other body compartments, with a suggested cutoff for sarcopenia of less than $1.6\,kg/m^2$ [43, 45]. In contrast to CT and MR imaging, DEXA cannot measure intramuscular fat, which can account for 5–15% of observed muscle mass in obese people [20].

Skeletal muscle cross-sectional imaging with CT or MR imaging is considered to be a gold-standard tool, but high cost, limited access to equipment, and concerns about radiation exposure (CT) limit their use for routine clinical practice [12]. Both techniques are highly reproducible and can assess muscle quality and quantity, and the accuracy is not affected by hydration status or fluid overload. With the help of a specific software, CT scan can quantify skeletal muscle index (SMI), which is the muscle area on a CT at the level of the third lumbar vertebra (L3) corrected for height (cm^2/m^2) [70]. Patients within the spectrum of NAFLD have no defined SMI cutoffs for sarcopenia, except for those with end-stage liver disease. Additional CT-based measures include psoas muscle diameter and area, which require no specialized computer software. There are some conflicting data on the significance of this parameter; some authors describe its good ability to predict a 1-year post-transplantation mortality [71] or mortality on the liver transplantation waiting list, independently of MELD [72], while others question its representativeness and capacity to identify patients with higher waitlist mortality in cirrhosis [73, 74]. CT has the additional ability to determine muscle radiation attenuation (MRA, expressed in Hounsfield Units), a measure of muscle quality which is inversely related to muscle fat content [75]. It has been shown that diabetes mellitus is associated with a lower muscle mass and a reduced MRA [76, 77]. Furthermore, myosteatosis has a role in decreasing skeletal muscle mass in patients with chronic liver disease [78].

3.2. Assessment of Muscle Strength. Handgrip strength (HGS) is the most widely used method for determining muscle strength, with a good correlation with leg strength and most relevant outcomes [2, 3, 12]. HGS is currently recommended by both recent international guidelines (European Association of Study of Liver (EASL); European Society for Clinical Nutrition and Metabolism (ESPEN)) in the assessment of all patients with cirrhosis and liver failure [79, 80]. It is usually performed with a calibrated dynamometer using the nondominant hand and averaged after three successful attempts. Patients with NAFLD have been shown to have higher odds for low muscle strength on HGS measurements irrespective of sociodemographic characteristics, weight, metabolic syndrome, and concurrent illnesses [81]. In patients with cirrhosis, studies have confirmed a correlation between decreased HGS and increasing mortality [82, 83].

3.3. Assessment of Physical Performance. A number of tests evaluating the physical performance can be used in the assessment of sarcopenia. Short physical performance battery (SPPB) was initially developed in geriatric population and assessed balance, gait, strength, and endurance by examining an individual's ability to stand with the feet together in side-by-side, semitandem, and tandem positions, time to walk 8 ft, and time to rise from a chair and return to the seated position five times, each scored out of 4 [84]. The SPPB allows for risk stratification and classifies the performance as low (0–6), intermediate (7–9), or higher performance (10–12), with the cutoff point for the diagnosis of sarcopenia in the elderly ≤8. Even though data are lacking in NAFLD, a score <10 increases the odds of mortality by 2.5 in patients with cirrhosis [61]. In 2017, Lai and coworkers developed "Liver Frailty Index (LFI)" for the assessment of muscle strength and function in liver disease. The LFI consists of dominant HGS, time to do 5 chair stands and

Table 1: Techniques and criteria for assessing muscle mass, muscle strength, and physical performance.

	Sarcopenia criteria	Assessment technique	Adjustment	Cutoff values	
				Men	Women
Muscle mass	EWGSOP [12], FNIH [87]	DXA	ASM	<20 kg	<15 kg
		DXA	ASM/height^2	<7.0 kg/m^2	<5.5 kg/m^2
		BIA	Predicted skeletal muscle mass equation (SM/height^2)	<8.87 kg/m^2	<6.42 kg/m^2
	AWGS [88]	BIA	ASM/height^2	<7.0 kg/m^2	<5.7 kg/m^2
		DXA	ASM/height^2	<7.0 kg/m^2	<5.4 kg/m^2
	FNIH [87]	DXA	ASM/BMI	<0.789 kg/BMI	<0.512 kg/BMI
	NAWGSLT [44]	CT	SMI	<50 cm^2/m^2	<39 cm^2/m^2
Muscle strength	EWGSOP [12]	Handgrip strength		<27 kg	<16 kg
	AWGS [88]			<28.0 kg	<18.0 kg
	FNIH [87]			<26 kg	<16 kg
Physical performance	EWGSOP [12], AWGS [88]	Gait speed	4-m course	≤0.8 m/s	
		SPPB		≤8 point score	

∗EWGSOP, The European Working Group on Sarcopenia in Older People; AWGS, The Asian Working Group for Sarcopenia; FNIH, The Foundation for the National Institutes of Health; NAWGSLT, North American Working Group on Sarcopenia in Liver Transplantation; DXA, dual energy X-ray absorptiometry; BIA, bioimpedance analysis; ASM, appendicular skeletal mass; SMI, skeletal muscle index; CT, computerized tomography; SPPB, short physical performance battery.

time holding 3 balance positions (feet side-by-side, semi-tandem, and tandem), and result in a continuous variable that can then be categorized into frail, prefrail, and robust and assessed longitudinally [85]. The LFI has been shown to be a good predictor of both pre- and postliver transplant morbidity and mortality, independent of the severity of the underlying liver disease [85, 86]. As in the case of previous tests, the LFI has not been validated in patients without cirrhosis.

In Table 1, there are techniques and criteria for assessing muscle mass, muscle strength, and physical performance.

4. Clinical Data Linking NAFLD and Sarcopenia

Most data connecting NAFLD and sarcopenia come from studies on Asian population, even though studies on Caucasians are also emerging. Most of the studies are published in the last 6 years. After adjustment for confounding factors, most data confirm direct interaction between NAFLD and sarcopenia (Table 2).

In 2014, Hong et al. [32] analyzed 452 participants. NAFLD was diagnosed by liver attenuation index (LAI), obtained by abdominal computed tomography (CT). Sarcopenia was defined by skeletal muscle mass index (SMI) that was obtained by dual energy X-ray absorptiometry (DXA). SMI had a negative correlation with hs-CRP, triglycerides, HOMA-IR, and with total body fat. Patients who had lower muscle mass had more than five times the higher risk of NAFLD even after adjusting for potential confounding determinants [32]. Additionally, study of Lee et al., on subjects from Korean National Health and Nutrition Examination Surveys, indicate a positive association of NAFLD and sarcopenia regardless of MetS and obesity [35]. Similar data were reported by Kim et al. [89] in 3739 Korean patients in whom NAFLD was defined by fatty liver index (FLI) in the absence of other CLD, but in their study, the association was different with respect to the age group and menopause status. Hashimoto et al. [90] analyzed the relationship of liver steatosis and SMI in 145 Japanese patients with T2DM. NAFLD was defined by trainset elastography (TE) with the controlled attenuation parameter (CAP). In this study, SMI showed a significant negative correlation with liver steatosis defined by CAP values, but only in men participants with T2DM. Interestingly, authors have showed that a 1% increment in SMI was associated with a decreased risk for steatosis by 20% in men with T2DM. Wijarnpreecha et al. [91] in their cross-sectional study investigated data of 11325 US participants. NAFLD was defined by US and sarcopenia with the help of BIA. Authors had reported that sarcopenia was an independent predictor of NAFLD and fibrosis [91]. Interesting data were published by Meng et al. [93] where authors analyzed the association between NAFLD and grip strength (GS), which was measured by an electronic handgrip dynamometer in a large population of 20957 Chinese participants. NAFLD was defined by abdominal US. Authors had reported that GS is negatively associated with NAFLD [93]. In these studies, NAFLD was defined by noninvasive methods; however, still the gold standard for NAFLD diagnosis and grading is liver biopsy. Liver biopsy is especially important in terms of differentiation of nonalcoholic fatty liver or simple steatosis from the necroinflammatory form of NAFLD (i.e., NASH). More convincing data are coming from the study of Koo et al. [33]. In this study, NAFLD was defined by liver biopsy in a large cohort of 309 patients. Authors had clearly showed that the prevalence of sarcopenia was related to the severity of NAFLD. Moreover, those participants with sarcopenia had an increased risk for NASH (OR 2.30; 95% CI 1.08–4.93) and significant fibrosis (OR 2.05; 95% CI 1.01–4.16), respectively. These associations were independent of IR and obesity [33]. Similar data were published in 255 Western patients with NAFLD [94] where NAFLD was also defined by liver histology. All of these studies had cross-sectional design; thus, the causal relationship could not be investigated. In the

Table 2: Clinical studies linking NAFLD and sarcopenia.

Author and year of publication	Study population	Study design	Method of NAFLD detection	Method of sarcopenia detection	Results
Hong et al. 2014 [32]	452 Korean participants	Cross-sectional	CT	DXA	Patients who had lower muscle mass had more than 5 times higher risk of NAFLD
Lee et al. 2016 [35]	2761 Korean participants	Cross-sectional	NAFLD liver fat score, CNS, HSI. Fibrosis by NFS, FIB-4, and Forns index	DXA	Sarcopenia was related to the significant fibrosis. This association was independent of obesity and insulin resistance.
Kim et al. 2016 [89]	3739 Korea participants	Cross-sectional	FLI	DXA, SMI	Low SMI was associated with FLI (i.e., NAFLD)
Hashimoto et al. 2016 [90]	145 Japanese patients with T2DM	Cross-sectional	TE with CAP	DXA, SMI	SMI had negative correlation with CAP values in men participants with T2DM. A 1% increment in SMI was associated with a decrease risk for steatosis by 20% in men with T2DM.
Wijarnpreecha et al. 2019 [91]	11325 US participants	Cross-sectional	US	BIA	Sarcopenia was an independent predictor of NAFLD and fibrosis
Lee et al. 2019 [92]	4398 Korea participants	Retrospective	US	BIA	An increase in fat mass and a loss of appendicular skeletal mass with aging were associated with incident NAFLD
Meng et al. 2016 [93]	20957 Chinese participants	Cross-sectional	US	Dynamometer	GS is negatively associated with NAFLD
Koo et al. 2017 [33]	309 Korean participants	Cross-sectional	Liver biopsy	BIA	The prevalence of sarcopenia was related to the severity of NAFLD; participants with sarcopenia had increased risk for NASH (OR 2.30; 95% CI 1.08–4.93) and significant fibrosis (OR 2.05; 95% CI 1.01–4.16), respectively
Petta et al. 2017 [94]	255 Italian participants	Cross-sectional	Liver biopsy	BIA	Sarcopenia independently associated with the severity of steatosis and fibrosis on liver histology
Kim et al. 2018 [24]	13165 Korean participants	Prospective	HSI	BIA	Increases in skeletal muscle mass over time had a beneficial effect in terms of NAFLD development and in terms of the resolution of existing NAFLD
Peng et al. 2019 [65]	2551 US participants	Cross-sectional	US	SMI—calculated as the absolute muscle mass (kg) divided by height^2 (meters) or total body mass (kg)	Steatosis defined by US was related to a decreased risk of sarcopenia when it is defined by height-adjusted SMI. Severe US defined steatosis was related to an increased risk of sarcopenia when sarcopenia is defined by the weight-adjusted SMI

∗NAFLD, nonalcoholic fatty liver disease; CT, computerized tomography; FLI, fatty liver index; DXA, dual energy X-ray absorptiometry; CNS, comprehensive NAFLD score; NFS, NAFLD fibrosis score; HIS, hepatic steatosis index; SMI, skeletal muscle index; TE, transient elastography; CAP, controlled attenuation parameter; US, ultrasound; BIA, bioimpedance analysis; GS, grip strength.

longitudinal study published two years ago, authors analyzed 10534 participants without baseline NAFLD and 2631 participants with baseline NAFLD [24]. NAFLD was defined by hepatic steatosis index (HIS) and sarcopenia by bioelectrical impedance analysis (BIA) [24]. The follow-up period was 7 years. Authors had found that increases in

skeletal muscle mass over time had a beneficial effect in terms of NAFLD development and in terms of the resolution of existing NAFLD [24]. As it is clearly shown, most of studies had shown a significant correlation between the NAFLD and sarcopenia. However, opposite data are coming from two recent studies. Peng et al. [65] analyzed 2551 US patients in whom NAFLD was defined by ultrasound. The definition of sarcopenia included both a low muscle mass and poor function. The skeletal muscle index (SMI) was calculated as the absolute muscle mass (kilograms) divided by height^2 (meters) or total body mass (kilograms). Authors reported that liver steatosis defined by US was related to a decreased risk of sarcopenia when it is defined by height-adjusted SMI. On the other hand, severe US-defined steatosis of the liver was related to an increased risk of sarcopenia when sarcopenia is defined by the weight-adjusted SMI. Authors conclude that definition of sarcopenia is important when we investigate the relationship among sarcopenia and NAFLD [65]. Additionally, Zhai et al. [95] failed to show the association among NAFLD and sarcopenia. Taking together all these data, the relationship of sarcopenia with visceral obesity and IR seems as an important risk factor for NAFLD, which further accelerates NAFLD progression to more advanced stages of CLD. However, prospective studies are needed that will give us information regarding the causal effect of NAFLD and sarcopenia.

5. Further Directions

NAFLD and sarcopenia share many of the determinants involved in their pathogenesis, most importantly, IR and chronic inflammation. Because of the overlap in the pathogenesis of sarcopenia and NAFLD, there are still many open questions. First, overlap in the pathogenesis makes it challenging to determine whether sarcopenia is just a complication of NAFLD or risk factor for NAFLD development and progression to more severe stages such as NASH and fibrosis. Due to the fact that currently published studies clearly confirm the relationship of sarcopenia with the degree of NAFLD defined and by liver histology, there is no doubt that the connection between these two entities exists, some even independent of MetS and IR. However, since most of the studies that investigated the relationship between NAFLD and sarcopenia are cross-sectional, the causality still cannot be drawn with certainty. Thus, further prospective studies that will give us an answer if sarcopenia is a consequence or a risk factor for NAFLD are warranted. Second, if the research proves that sarcopenia is a risk factor, treatment strategies such as physical exercise and nutritional supplementation that are dominantly a part of sarcopenia management should be investigated in the context of NAFLD. In other words, given the fact that for now, we do not have a good treatment option for NAFLD, research should combine studies on NAFLD and sarcopenia in terms of physical activity and nutritional interventions such as supplementation of vitamin D. With this approach, we might see the possible effect of the sarcopenia treatment on NAFLD. This is important not only in the context of sarcopenia and NAFLD but also in the context of NAFLD as a multisystemic disease. Recently, Han et al. [18] had showed that patients with both NAFLD and sarcopenia had a higher risk for atherosclerotic cardiovascular disease (OR = 1.83, $P = 0.014$) compared with those without NAFLD and sarcopenia. Thus, studies that will involve also extrahepatic manifestations of NAFLD joined with sarcopenia would be of great interest. Third, the role of myokines is the most attractive in the context of sarcopenia because additional knowledge of their role could provide an effective medication which might treat both NAFLD and sarcopenia. Fourth, myosteatosis can have a greater influence on muscle function than muscle mass itself. Thus, it would be interesting to investigate whether myosteatosis is linked to increased morbidity and mortality in the population of NAFLD patients. Fifth, by definition, sarcopenia includes all three components: loss of skeletal muscle mass, strength, or physical performance. According to current guidelines, muscle function is a main determinant in the evaluation of sarcopenia. However, methods for its assessment are not well investigated in the context of NAFLD, which consequently may lead to a lower detection rate of sarcopenia. Therefore, further investigations on the effect of the low muscle function/performance on development and progression of NAFLD are warranted. Sixth, we need studies that will investigate what is the optimal method for detection of loss of skeletal muscle mass, strength, and physical performance in the context of NAFLD. These methods should also be patient-friendly, noninvasive, uncostly, and available in everyday clinical practice.

Data Availability

The data used to support this study are included within this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors contributed equally to this review. All authors have read and agreed to the published version of the manuscript.

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Research Article

Characteristics of NAFLD Based on Hypopituitarism

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Background. Hypopituitarism and hypothalamic disorders, which induce central obesity and appetite disorder, are associated with nonalcoholic fatty liver disease (NAFLD). We retrospectively analyzed the clinical features of NAFLD patients with hypopituitarism. *Patients*. We examined the cases of 15 NAFLD patients with hypopituitarism (mean age, 39.4 years; males/females, 11/4). The causes of hypopituitarism were surgical in eight cases (six with craniopharyngioma and two with prolactinoma) and nonsurgical in seven cases, including unexplained hypopituitarism in five cases, Sheehan syndrome in one case, and one case that occurred after the radiation therapy. Serum adiponectin, soluble tumor necrosis factor receptor-2 (TNFR-2), and leptin levels were measured. *Results*. We compared the cases of the eight patients who underwent cranial surgery due to craniopharyngioma or prolactinoma and seven nonsurgical cases. The body mass index (surgery group, 30.2 ± 4.1; nonsurgery group, 29.2 ± 14.2) and the rate of diabetes (75% in surgery group, 14.3% in nonsurgery group) tended to be higher in the surgery group, and the hepatic fibrosis grade (surgery group, 3.75 ± 0.38; nonsurgery group, 1.64 ± 1.07) was significantly higher in the surgery group. The levels of adipocytokines, serum adiponectin, and serum soluble TNFR-2 showed no correlation with hepatic fibrosis, whereas the serum leptin levels were significantly correlated with liver fibrosis ($R = 0.696$). *Conclusion*. The hepatic fibrosis grade rapidly progressed in the cranial surgery cases of NAFLD patients with hypopituitarism, possibly in association with BMI, diabetes mellitus, and leptin. In such cranial surgery patients, strong interventions should be considered from the early stage, including diet education, hormone replacement, and more.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) has become the most common type of liver disease in developed countries worldwide. NAFLD covers a wide spectrum from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis and hepatocellular carcinoma [1–3]. NAFLD is closely associated with obesity, lifestyle, and lifestyle-related diseases [1–5].

Hypopituitarism, resulting in central obesity, is frequently known to lead to a condition similar to metabolic syndromes such as hyperlipidemia, impaired glucose tolerance, and fatty liver [6]. It has been reported that in patients with NAFLD and lifestyle-related diseases based on growth hormone (GH) deficiency associated with hypopituitarism, these metabolic conditions and liver function were improved by the administration of GH [7]. In addition, thyroid dysfunction is observed in approximately 25% of patients with NAFLD [8]. These findings suggested that endocrine hormonal abnormalities are closely related to the development of NAFLD.

The hypothalamus, where leptin receptors are present, is closely related to appetite control and to adjustments of the sympathetic nervous system, and thus, the relationship between hypothalamic disorders and NAFLD is also attracting attention [9]. These findings suggested that hypopituitarism and hypothalamic disorders are closely related to the development of NAFLD.

Based on the above reports, we examined the clinical features of NAFLD patients with hypopituitarism and/or hypothalamus disorder.

2. Patients and Methods

2.1. Patients. We collected the data of the 15 patients with NAFLD with hypopituitarism who were diagnosed based on findings obtained by a liver biopsy, blood test, endocrine test, CT scan, or other modes between January 2000 and December 2019 at our institute for a retrospective analysis. This study conformed to the ethical guidelines of the Declaration of Helsinki (2000 version) and was approved by our institute's ethics committee. Regarding informed consent, if we were able to contact the patients, we obtained their informed consent directly. For the other cases, we posted the study plan on our institute's home page. If any patients or bereaved family members refused their consent for this study project, we deleted the patient's data.

2.2. Diagnosis and Management of NAFLD with Hypopituitary Dysfunction. The diagnosis of NAFLD was based on the following criteria: (1) the detection of hepatic steatosis (or steatohepatitis) by liver biopsy (steatosis was diagnosed in >5% of liver biopsies [10]); (2) ethanol intake <20 g/day in women or <30 g/day in men (confirmed by the attending physician and/or family members in close contact with the patient); and (3) appropriate exclusion of other liver diseases. Other liver diseases, such as alcoholic liver disease, viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, biliary obstruction, and metabolic liver diseases such as Wilson's disease and hemochromatosis were excluded on the basis of patient interviews regarding ethanol intake, blood chemistry tests, virus markers, auto-antibody, ultrasound, and CT scans [2, 10].

The diagnoses of hypopituitarism and hormone replacement therapy were performed by the department of medicine II, endocrinology, and hypertension at our hospital. Briefly, several tests were performed for the diagnosis of hypopituitarism: (i) blood findings, thyroid hormone levels, and adrenal and sex hormones were measured. (ii) Several hormone stimulation tests matched to the patient's hormone levels were conducted (TRH stimulating test and ACTH stimulating test). (iii) The results of brain imaging magnetic resonance imaging (MRI) or high-resolution CT and pituitary tumor or other pituitary gland problems were examined. (iv) Vision tests: we investigated whether each patient's sight or visual field was impaired. In some patients' cases, to compensate for hormone deficiencies, hormones such as GH, glucocorticoid, thyroid hormone, antidiuretic hormone, and/or sex hormones were administered, matched to the individual patient's hormone level.

The date of the liver biopsy was taken as the time of the patient's diagnosis of NAFLD. Each patient's body mass index (BMI), liver enzymes, and lipid and glucose profiles were obtained at the time of liver biopsy thereafter. The diagnosis of type II diabetes mellitus was based on the World Health Organization criteria. Liver histology was reviewed by liver pathologists and was evaluated according to the modified classification published by Brunt [11, 12]. Fibrosis was scored using a five-grade scale: F0, normal connective tissue; F1, perivenular or pericellular fibrosis in zone 3; F2, perivenular or pericellular fibrosis with focal or extensive portal/periportal fibrosis; F3, bridging or septal fibrosis; and F4, cirrhosis. The inflammatory grade and steatosis grade were also evaluated.

After the liver biopsy, enzyme-linked immunosorbent assays were used to measure the patient's serum levels of adiponectin (Otsuka Pharmaceutical, Tokyo), soluble tumor necrosis factor receptor-2 (TNFR-2, Biosource Europe, Fleurus, Belgium), and leptin (AssayPro, St. Charles, MO, USA).

2.3. Statistical Analyses. The patients' data were analyzed with SPSS version 13.0J software (SPSS, Tokyo). The results are shown as median values or percentages. The Mann–Whitney test or chi-square test was performed to detect significant differences between the data of the cranial surgery NAFLD and nonsurgery NAFLD groups. The correlations between the hepatic fibrosis grade and the serum soluble TNFR-2 level, serum adiponectin level, and serum leptin level were examined by Spearman's correlation test. The correlation index (R) was calculated. In all analyses, probability (p) values <0.05 were considered to indicate the significance.

3. Results

As shown in Table 1, the mean age of the total series of 15 patients at the time point of their liver biopsy was 39.4 years old and the male/female ratio was 11/4. The patient's mean age at the diagnosis of hypopituitary dysfunction was 18.5 years old, and the duration from the diagnosis of hypopituitarism to the liver biopsy was 20.7 years.

The causes of hypopituitarism are also shown in Table 1. There were eight surgery cases (six patients with craniopharyngioma and two with prolactinoma) and seven nonsurgery cases, including five patients with unexplained pituitary dysfunction, one patient with Sheehan syndrome, and one patient with hypopituitarism after the radiation therapy. Thyroid hormone supplementation therapy was administered to all 15 patients, glucocorticoids were given to 12 patients (80%), and five patients received GH (33%). Regarding the liver pathological findings, all 15 patients showed steatohepatitis. The fibrosis grade was 2.80 ± 1.33, the inflammation grade was 1.90 ± 0.63, and the steatosis grade was 2.10 ± 0.89.

Based on our finding that all eight surgery patients (with craniopharyngioma or prolactinoma) showed a fibrosis grade of F3 or F4, we compared the eight surgery patients and seven nonsurgery patients (Tables 2–4). Although there was no significant between-group difference in age or gender, the surgery group tended to have higher BMI values (surgery group 30.2 ± 4.1 vs. nonsurgery group 29.2 ± 14.2, $p = 0.072$) and a higher rate of diabetes (75% in the surgery

Table 1: Clinical features of NAFLD with hypopituitarism.

Case	Gender	Age at diagnosis of hypopituitarism (yrs)	Age at liver biopsy diagnosis (yrs)	Cause of hypopituitarism	Surgery	Hormone replacement therapy*
1	M	5	16	Craniopharyngioma	○	G, S, T, and A (+radiation)
2	M	6	18	Craniopharyngioma	○	S, T, and A
3	M	6	44	Craniopharyngioma	○	S, T (+gamma knife)
4	M	9	24	Idiopathic hypopituitarism	X	T and A
5	M	10	18	Transection of the pituitary stalk	X	G, S, and T
6	M	12	43	Germ cell tumor	X	S and T
7	M	12	50	Idiopathic hypopituitarism	X	G and T
8	F	13	39	Prolactinoma	○	S and T
9	F	19	55	Pituitary adenoma	○	T
10	M	25	49	Transection of the pituitary stalk	X	G, S, and T
11	F	33	36	Craniopharyngioma	○	S, T, and A (+gamma knife)
12	M	33	59	Sheehan syndrome	X	S and T
13	M	34	42	Craniopharyngioma	○	S, T, and A
14	M	58	75	Prolactinoma (+radiation)	○	S and T
15	M	2	23	Idiopathic hypopituitarism	○	G, S, and T

A, antidiuretic hormone; C, glucocorticoid; G, growth hormone; T, thyroid hormone.

Table 2: Comparison between surgical cases and nonsurgical cases.

	Surgical	Nonsurgical	p value
No.	8	7	
Female (%)	37.5%	4.3%	ns
Diagnosis age of hypopituitarism	16 ± 18.9	10.6 ± 10	ns
Diagnosis age of liver biopsy	40.3 ± 19.0	38.0 ± 16.1	ns
BMI	30.2 ± 4.1	29.2 ± 14.2	0.072
Obesity (BMI > 25)	87.5%	50%	0.067
Obesity (BMI > 30)	50%	14.3%	ns
Diabetes mellitus	75%	28.6%	0.072
Hypertension	50%	28.6%	ns
Dyslipidemia	75%	85.7%	ns

Data are mean ± SD or percentages. ns, not significant. BMI, body mass index.

Table 3: Comparison of blood tests between surgical cases and nonsurgical cases.

	Surgical	Nonsurgical	p value
Total bilirubin (mg/d)	0.7 ± 0.4	0.7 ± 0.3	ns
Albumin (g/dl)	3.9 ± 0.7	4.5 ± 0.3	0.054
AST (IU/L)	82 ± 39	94 ± 132	ns
ALT (IU/L)	72 ± 29	158 ± 258	ns
gGTP (IU/ml)	185 ± 64	156 ± 114	ns
Total cholesterol (mg/dl)	190 ± 27	241 ± 38	0.029
Triglyceride (mg/dl)	156 ± 64	227 ± 156	ns
Platelet (×104/ml)	13.6 ± 6.5	26.2 ± 6.0	0.002
Prothrombin time (%)	78.5 ± 13.3	89.3 ± 14.5	ns
HbA1C (%)	7.1 ± 2.5	5.4 ± 0.6	ns

Data are mean ± SD. ns, not significant.

group vs. 28.6% in the nonsurgery group, $p = 0.072$). The platelet count and serum cholesterol level were significantly lower in the surgery group. Regarding liver pathological findings, the fibrosis grade was significantly more severe in the surgery group at 3.75 ± 0.38 versus the nonsurgery group's grade at 1.64 ± 1.07 ($p = 0.01$). The activity grade and steatosis grade were not significantly different between the surgery and nonsurgery groups. We confirmed the abovementioned significant differences using a multivariate unconditional logistic regression model. The between-group differences in the hepatic fibrosis grade ($p = 0.01$), serum albumin level ($p = 0.048$), platelet count ($p = 0.04$), and total cholesterol level ($p = 0.012$) were confirmed to be significant.

Table 4: Comparison of liver pathological findings between surgical cases and nonsurgical cases.

	Surgical	Nonsurgical	p value
Fibrosis (F0–F4)	3.75 ± 0.38	1.64 ± 1.07	0.01
Inflammation (A0–A3)	1.17 ± 0.49	2.06 ± 0.73	ns
Steatosis (S0–S3)	2.31 ± 0.88	1.86 ± 0.90	ns

Data are mean ± SD. ns, not significant.

For our investigation of the associations of serum adiponectin values and serum leptin values with serum soluble TNFR-2 levels, we examined 10 of the 15 patients' values and determined their correlation with liver fibrosis. The results revealed no correlation of the serum adiponectin level or the serum soluble TNFR-2 levels with the hepatic fibrosis grade. In contrast, these patients' serum leptin levels showed a significant correlation with their liver fibrosis grades ($R = 0.696$, $p = 0.025$) (Figures 1–3).

4. Discussion

The importance of NAFLD due to hypopituitarism has been described, as such cases of NAFLD can rapidly progress to cirrhosis even at a young age [13–15]. We have treated a

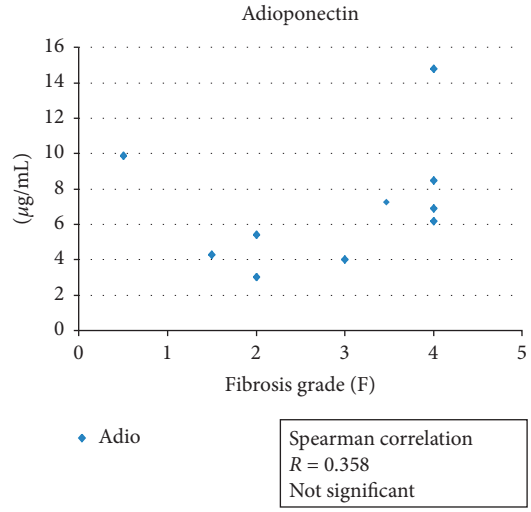

Figure 1: The association between the serum adiponectin level and hepatic fibrosis grade in 10 NAFLD patients with hypopituitarism. Spearman's correlation was obtained. There was no significant association ($R = 0.358$).

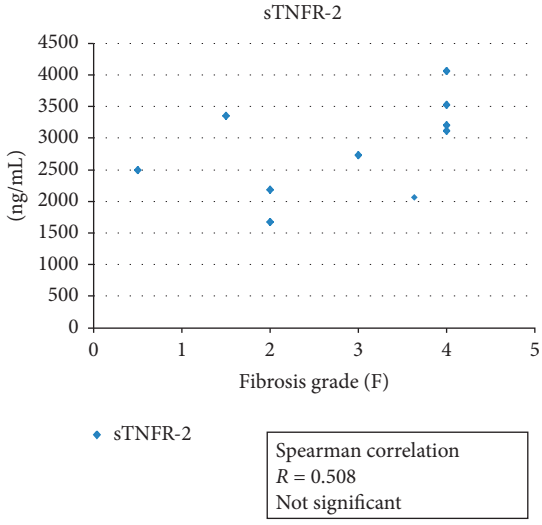

Figure 2: The association between the patients' serum soluble TNFR-2 level and hepatic fibrosis grade ($n = 10$). Spearman's correlation was obtained. There was no significant association ($R = 0.508$).

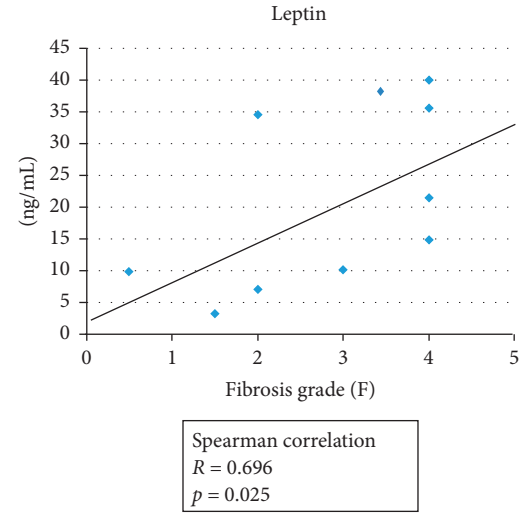

Figure 3: The association between the patients' serum adiponectin level and hepatic fibrosis grade ($n = 10$). Spearman's correlation was obtained. A significant association was observed ($p = 0.025$, $R = 696$).

number of these cases. In cases of GH deficiency associated with hypopituitarism, the prevalence of liver fat deposition in the abdominal echo is generally approximately 60%; the frequency of fatty liver is high compared to that in general populations [6]. Growth hormone was administered to the patients with lifestyle-related disease, and it enhanced their metabolism and energy consumption [6, 7]. The improvement of liver function after GH administration is considered to be due to improvements in the patient's metabolic and energy consumption state [7, 16]. Growth hormone is also expected to be useful as a treatment strategy aimed at the metabolic and energy improvement of general NAFLD patients in the future. A multicenter trial was performed to investigate the efficacy and safety of tesamorelin (a synthetic form of GH-releasing hormone) in NAFLD patients with HIV, and it was reported that tesamorelin treatment resulted in a greater reduction of the hepatic fat fraction [17].

In our present liver biopsy cases—specifically in the patients who underwent surgery due to craniopharyngioma or prolactinoma—it became clear that liver fibrosis had progressed. We speculate that this progression of liver fibrosis impaired not only the pituitary gland but also the hypothalamus, due to the presence of a large tumor or surgical resection. As the hypothalamus is involved in the appetite center and in the adjustments of the sympathetic nervous system, it is known that eating disorders and decreased sympathetic nerve activity are induced in addition to GH deficiency. The BMI values of our present surgery cases tended to be higher than those of the nonsurgery group, suggesting that the surgery patients' cases might have been associated with eating disorders due to a hypothalamus disorder.

In addition, leptin is produced by increased fat cells due to obesity, and it usually works in the hypothalamus to control the feeding center. However, in the present surgical cases, leptin would not have been functioning in the hypothalamus or feeding center. Ikejima et al. reported that leptin enhanced liver fibrosis [18, 19], and we thus speculate that in the present study's patients who underwent surgery, leptin caused the liver fibrosis to rapidly progress without appetite control. The significant correlation that was revealed between the serum leptin levels and liver fibrosis supports this speculation. Therefore, in these cranial surgical cases involving the pituitary gland and hypothalamus, the appearance of NAFLD had to be closely monitored. We recommend extensive diet therapy and hormone replacement therapy from an early stage for such patients.

We also measured other adipokines (i.e., adiponectin and soluble TNFR-2 instead of TNFα). Several papers have

demonstrated that both of these adipokines have an important role in progression and liver fibrosis in NASH/NAFLD [20–22]. In addition, Bach et al. reported that pituitary function is decisive for the catabolic response to TNFα [23]. Ames dwarf mice (which are GH-deficient) showed a beneficial adipocytokine profile, characterized by increased adiponectin and decreased proinflammatory cytokine (TNFα and interleukin-6) levels [24]. We therefore measured the present patients' levels of these two adipokines; however, we could not observe a significant correlation between the liver histological findings in NAFLD based on hypopituitarism.

We reported the case of the present patient #15 with idiopathic hypopituitarism and hepatopulmonary syndrome due to NASH [25]. After the administration of GH, the patient's NASH and hepatopulmonary syndrome were improved. Neither an appetite disorder nor obesity was observed in this patient, suggesting that his hypothalamus was intact and that the GH was thus effective. The treatment of patients with hypothalamic disorders might require another method in addition to the GH hormone therapy. In fact, it has been difficult to enforce dietary restrictions with normal dietary guidance for these patients. The elucidation of a new central appetite system other than the leptin-hypothalamic system and the development of an appetite suppression mechanism are expected.

Bariatric surgery is now also considered a treatment option for patients with NAFLD who find it difficult to control their appetite and body weight [26]. In our present patient series, bariatric surgery might have been an effective therapy method for appetite control; however, the safety and efficacy of bariatric surgery for patients with NASH cirrhosis have not been established. Even in NAFLD cases associated with metabolic syndrome, the involvement of glucocorticoids, GH, thyroid hormones, and a loss of appetite control, i.e., so-called leptin resistance, should be considered.

This study has several limitations. It was a single-center retrospective analysis of a small number of patients ($n = 15$). Multicenter and prospective studies are desired for the further elucidation of the findings obtained herein. In conclusion, hepatic fibrosis progresses rapidly in NAFLD patients with not only hypopituitarism but also hypothalamus dysfunction, and this might be associated with BMI, diabetes mellitus, and leptin.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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