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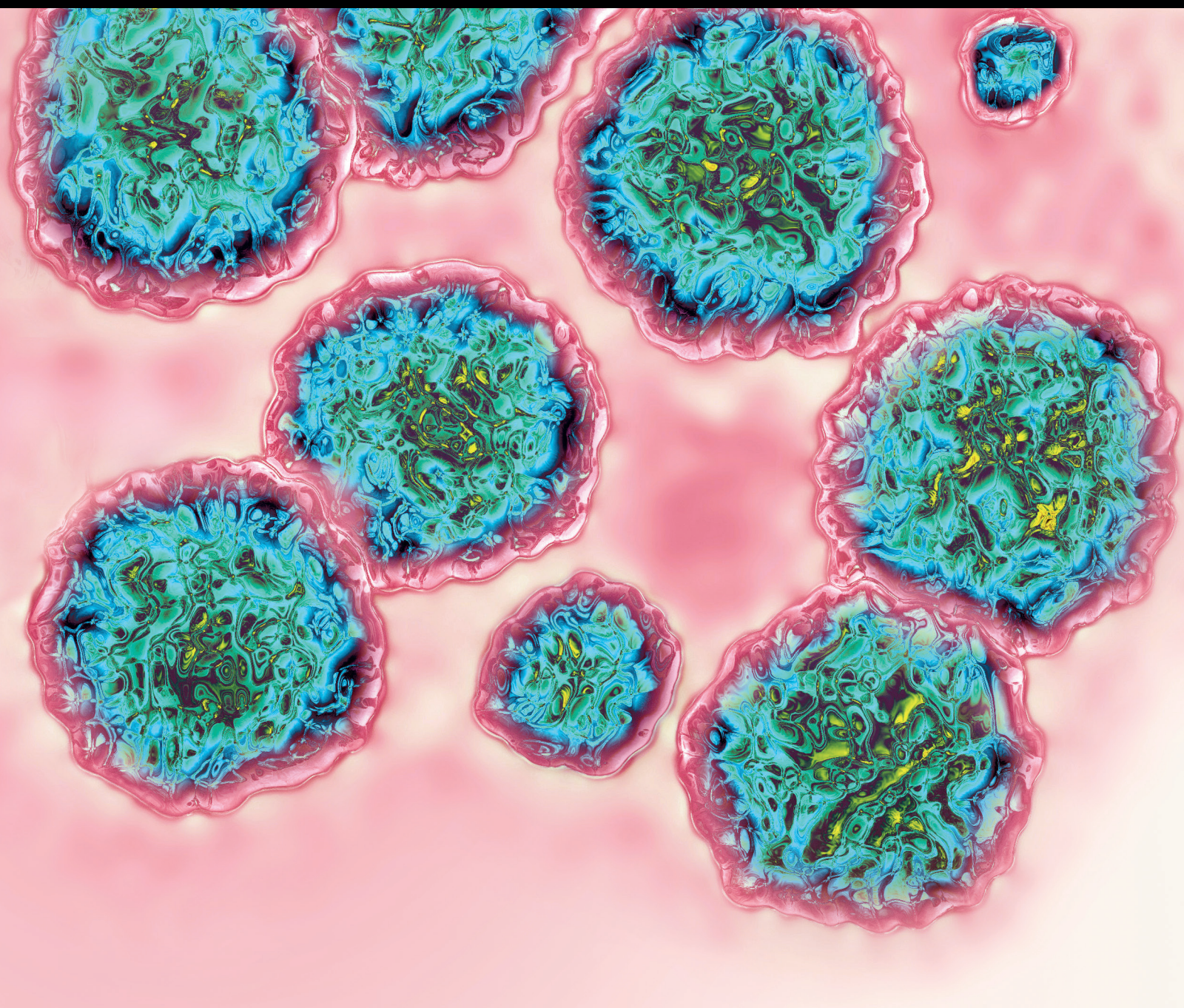
Chronic Viral Hepatitis and Metabolic Syndrome/Cardiovascular Risk

Lead Guest Editor: Peter Jarčuška
Guest Editors: Ahmed Abdel-Razik, Robert Flisiak, and Ram B. Singh



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Editorial Board

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Editorial

Chronic Viral Hepatitis and Metabolic Syndrome/Cardiovascular Risk

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Chronic viral hepatitis B and C can lead to liver cirrhosis. Hepatocellular carcinoma (HCC) is another complication, however, and, particularly interestingly, it can occur not only in patients with advanced liver fibrosis or cirrhosis, but also in patients with chronic hepatitis B infection and high viremia. Both decompensated liver cirrhosis and HCC lead to death in patients with chronic viral hepatitis B or C. There are several factors, including hepatic comorbidities such as nonalcoholic fatty liver disease (NAFLD) or alcoholic liver disease, which accelerate liver fibrosis in patients with chronic viral hepatitis [1].

Metabolic syndrome (MetS) is associated with insulin resistance. MetS is present more frequently in obese patients or in patients with type 2 diabetes mellitus (T2DM). Patients with MetS have significantly higher both cardiovascular morbidity and mortality compared to those without MetS. MetS is present in approximately 25% of population in advanced countries, while its prevalence demonstrates increasing trend with higher age [2]. MetS prevalence depends on many factors, including viral infection.

Cardiovascular risk is not higher in patients with chronic viral hepatitis B, nor is the risk of hyperlipidemia, T2DM, or MetS. On the other hand, MetS or NAFLD comorbidities accelerate liver fibrosis and increase HCC incidence among patients with viral hepatitis B [3].

Patients with chronic viral hepatitis C are being diagnosed more frequently with higher insulin resistance, prediabetes, and T2DM. T2DM occurrence is rising with stage of liver fibrosis and is very high in patients who failed to reach sustained viral response (SVR) after antiviral therapy was completed [4, 5]. Liver steatosis is present more frequently in patients with chronic hepatitis C. Viral steatosis is specific condition characterized by higher hepatocellular fat accumulation without higher insulin resistance in patients infected mainly with genotype 3 [5]. MetS prevalence is not higher in population of patients infected with hepatitis C virus, which has both higher T2DM prevalence and insulin resistance, compared to population of never infected patients. The reason is most likely that chronic hepatitis C patients do not have atherogenic hyperlipidemia [6]. Increased peripheral and hepatic insulin resistance, chronic inflammation, chronic endothelial injury, and direct viral effect on arterial wall most likely also lead to accelerated atherogenesis. Patients with chronic hepatitis C have higher cardiovascular risk compared to those never infected, together with increased prevalence of coronary artery disease, unstable angina pectoris, myocardial infarction, and stroke [7]. Interestingly, SVR achievement after interferon based treatment in patients with chronic hepatitis C reduced T2DM incidence in the future. Nevertheless, risk of T2DM development after SVR achievement is higher in patients with BMI>25 [4]. Nowadays, there is highly effective treatment of chronic hepatitis C. Nearly all patients achieve SVR by the treatment with direct acting antivirals (DAA); moreover, DAA therapy has very low occurrence of serious adverse events and is thus considered safe [8]. DAA therapy leads to both significant decrease in fasting glucose

(FG) levels and significant decrease in glycated hemoglobin levels (HbA1C) in diabetic patients with chronic hepatitis C and is thus requiring reduction of antidiabetic therapy in certain part of patients [9]. Successful DAA therapy will most likely lead to drop in both T2DM prevalence and cardiovascular risk, making it essential to remove all barriers limiting easy diagnostics and therapy. Patients with chronic viral hepatitis could also benefit from statin therapy for its antifibrotic and antineoplastic effect [10].

S. Drazilova et al. described in the review article pathophysiological mechanisms of both increased peripheral and hepatic insulin resistance. Authors also evaluated predictive factors of T2DM. Diabetes mellitus is present more frequently in patients with chronic hepatitis C and cirrhosis, while it also predicts liver decompensation. DAA treatment led to decrease in FG levels and/or HbA1C levels in nearly all studies, limitation of most of the studies was retrospective design. Approximately 3-40% of diabetic patients with chronic hepatitis C required reduction of antidiabetic therapy during DAA therapy. Patients with chronic hepatitis C rarely have elevated total cholesterol (TC), LDL cholesterol (LDL-C), and triglycerides (TG) levels. However, during DAA therapy, one can await alteration of lipoprotein profile. Studies observed elevation in both TC and LDL-C levels; on the other hand, most of the studies observed both drop in TG and elevation in HDL-C levels during DAA therapy, although, further research into alteration of lipoprotein profile during DAA therapy is still required.

Retrospective analysis from Slovakia (S. Drazilova et al.) evaluated glucose metabolism changes in patients with chronic hepatitis C treated with DAA. Altogether, 370 patients were observed, 45,9% in F4 by Metavir. Risk of T2DM development increases with liver fibrosis stage. T2DM was found in 14.4% patients with F0-F2 fibrosis, 21.3% patients with F3, and 31.8% with F4 fibrosis (p=0.004). FG levels, impaired fasting glucose (IFG) or T2DM prevalence were not significantly different between patients in Child-Pugh A and Child-Pugh B/C stage. Correlations between FG and APRI score were observed (R^2 = 0.018, p=0.026). Treatment experienced patients had significantly higher FG (p=0.006) and significantly higher prevalence of IFG or T2DM (p=0.005) when compared to treatment naive patients. Age, BMI, and F4 stage of fibrosis by Metavir predicted in univariate analysis T2DM prevalence. DAA treatment led to drop in FG in all patients (p=0.002), patients with IFG (p<0.0001), and T2DM (p<0.0001), although not in patients without IFG or T2DM (p=0.192). Significant drop in FG was observed in all experienced patients (p<0.0001), experienced cirrhotics (p<0.0001), although not in treatment naive patients. Drop in FG was observed in all cirrhotic patients (p=0.009), however not in patients with Child-Pugh score B/C (p=0.568). Significant change in FG levels during and after DAA therapy was not observed in patients with liver fibrosis F0-F3 by Metavir. Baseline FG was only predictor of significant decrease of glycemia (>5%) after DAA treatment (p<0.0001).

S. Mustapic et al. correlated ultrasound grade of liver steatosis with the risk of MetS. Authors evaluated 159 patients, 34% of them were obese. Patients with higher ultrasound grade of steatosis had significantly higher BMI, increased prevalence of obesity, impaired glucose metabolism, atherogenic dyslipidemia, raised blood pressure and significantly more frequently met the modified criteria of MetS (P<0.05 for all analyses). Ultrasound grade of liver steatosis was significantly independently associated with the presence of MetS (p=0.007 for moderate-to-severe liver steatosis). Authors did not find significant difference in FIB4 value in different ultrasound grades of liver steatosis (p=0.251).

M. Flisiak-Jackiewicz et al. studied predictive role of interleukin-18 (IL-18) in liver steatosis in obese children. IL-18 correlates with ALT (p=0.036), AST (p=0.032), GGT (p=0.016), TG (p=0.027), hs-CRP (p=0.014), ultrasound grade of liver steatosis (p=0.044), and waist circumference (p=0.009). Level of IL-18 was higher in obese children with advanced liver steatosis found on ultrasound examination compared to children without steatosis (p=0.027). The concentration of IL-18 was significantly higher in obese children with steatosis found on magnetic resonance proton spectroscopy (^{1}HMRS) compared to children without fatty liver (p=0.014). IL-18 will most likely be used as NAFLD predictor in obese children in the future.

T. Stroffolini et al. described characteristics and changes over time of alcohol-related chronic liver diseases in Italy. Authors enrolled 12 256 subjects from two national surveys made in 2001 and 2014. 2 717 (22.2%) cases had a risky alcohol intake; 48.3% of them were anti HCV positive. Sex ratio (male/female) decreased from 3.8 in 2001 to 1.3 in 2014 and women were significantly older than men (58.9 versus 53.1 years; p<0.01). The proportion of subjects with liver cirrhosis increased over time in both sexes, and decompensation of liver cirrhosis (Child B or C) was found in approximately half of cases. Risky alcohol drinking plays important role in chronic liver diseases in Italy.

Studies mentioned above highlight that the association between chronic hepatitis C and T2DM is a very serious medical issue, which is present mainly in advanced chronic hepatitis C, in patients with liver cirrhosis or in experienced patients. Furthermore, studies also highlight that DAA therapy leads to drop in FG. Liver steatosis and risky alcohol intake can eventually deteriorate clinical course of chronic viral hepatitis, making it necessary to diagnose all simultaneous hepatic comorbidities as early as possible, eventually to look for surrogate markers (such as IL-18) for liver fat accumulation diagnostics in patients at risk.

Conflicts of Interest

Peter Jarcuska reports personal fees and nonfinancial support from AbbVie and Gilead and personal fees from MSD, outside the submitted work. Ahmed Abdel-Razik, Robert Flisiak, and Ram B. Singh report no conflicts of interest.

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Robert Flisiak
Ram B. Singh

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Research Article

Glucose Metabolism Changes in Patients with Chronic Hepatitis C Treated with Direct Acting Antivirals

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Background and Aims. Chronic hepatitis C is a systemic disease and type 2 diabetes mellitus (T2DM) belongs to more common extrahepatic. The aim of this study was to (i) explore the prevalence of impaired fasting glucose (IFG) and T2DM in patients with chronic hepatitis C, (ii) explore the effect of direct acting antivirals (DAA) treatment on the glycemia, and (iii) explore the factors that modulate the effect of DAA treatment on glycemia in patients with chronic hepatitis C. *Methods*. We performed a longitudinal retrospective observational study focused on the patients undergoing DAA treatment of chronic hepatitis C. Data about glycemia, history of diabetes, hepatitis C virus, treatment, and liver status, including elastography, were obtained at baseline (before treatment start), at the end of treatment and 12 weeks after the end of treatment. Patients were treated with various regimens of direct acting antivirals. *Results*. We included 370 patients; 45.9% had F4 fibrosis. At baseline, the prevalence of T2DM increased with the degree of fibrosis (F0-F2 14.4%, F3 21.3%, and F4 31.8%, p=0.004). Fasting glycemia also increased with the degree of fibrosis (F0-F2 5.75±0.18 F3 5.84±0.17, and F4 6.69±0.2 mmol/L, p=0.001). We saw significant decrease of glycemia after treatment in all patients, but patients without T2DM or IFG from 6.21±0.12 to 6.08±0.15 mmol/L (p=0.002). The decrease was also visible in treatment experienced patients and patients with Child-Pugh A cirrhosis. *Conclusion*. We confirmed that the prevalence of either T2DM or IFG increases in chronic hepatitis C patients with the degree of fibrosis. The predictive factors for T2DM were, besides F4, fibrosis also higher age and BMI. Significant decrease of fasting glycemia after the DAA treatment was observed in the whole cohort and in subgroups of patients with T2DM, IFG, cirrhotic, and treatment experienced patients.

1. Introduction

About 170 million people were infected with Hepatitis C virus (HCV) in 2013. Overall prevalence is slightly decreasing, mainly due to effective treatment [1]. Active viral replication of HCV is present in about 70 million people worldwide [2]. Chronic hepatitis C may progress to liver cirrhosis. Hepatocellular cancer (HCC) usually occurs in bridging fibrosis (Metavir F3) and cirrhosis (Metavir F4). Decompensated liver cirrhosis and hepatocellular cancer are two most common causes of death of patient with chronic hepatitis C [3].

The treatment by direct acting antivirals (DAA), which inhibit NS3/4a protease and NS5A and NS5B polymerase, leads to sustained virological response (SVR) in almost all infected patients [4]. Achievement of SVR decreases liver-related as well as all-cause mortality in these patients [5].

Chronic hepatitis C is a systemic disease, because it damages also other organs besides liver. Almost three quarters of patients with chronic hepatitis C have extrahepatic manifestations. These may develop well before the diagnosis of chronic hepatitis C [6]. Type 2 diabetes mellitus (T2DM) belongs to more common extrahepatic manifestations of chronic hepatitis C [7]. Insulin resistance is significantly more common in patients with chronic hepatitis C, even with low degree fibrosis, compared to healthy controls. Insulin resistance is also associated with fibrosis progression and portal inflammation [8]. T2DM is significantly more common in patients with HCV related cirrhosis compared to noncirrhotic patients and in chronic hepatitis C patients who failed interferon-based treatment [9]. T2DM is also associated with more frequent occurrence of HCC in patients with chronic hepatitis C [10]. On the other hand, prevalence of HCV infection is higher among T2DM patients compared to nondiabetic patients [11].

About 347 million people world-wide are diagnosed with T2DM [1] and the prevalence is increasing. Fifty-six million people suffer from T2DM in Europe only and estimated prevalence is 8.5% [12]. T2DM is associated with lower life expectancy based on the age of diagnosis [13].

The aim of this study was to

(1) explore the prevalence of Impaired fasting glucose (IFG) and T2DM in patients with chronic hepatitis C and various degrees of liver fibrosis

(2) explore the effect of DAA treatment on the glycemia levels in patients with chronic hepatitis C

(3) explore the factors that modulate the effect of DAA treatment on glycemia in patients with chronic hepatitis C

2. Patients and Methods

We designed a retrospective longitudinal observational study focused on the patients undergoing DAA treatment of chronic hepatitis C in multiple centres in Slovakia.

Ethics committee of Poprad Hospital, Banícka 803/28, 05845 Poprad, Slovakia, approved the biomedical research protocol (no. 14/5/2018). Only retrospective anonymized patient data were used; patients signed general informed consent for usage of these data outside standard clinical care. The study was performed in compliance with the Declaration of Helsinki.

2.1. Patients. Study included consecutive patients treated for chronic hepatitis C in multiple centres in Slovakia. Exclusion criteria were age less than 18 years, noncompliance, and malignancy with the exception of hepatocellular cancer and localised malignancies of the skin. All patients were treated with standard of care DAA treatment according to guidelines valid at the time of the treatment. DAA treatment regimens included (i) ombitasvir, paritaprevir, ritonavir, and dasabuvir (3D combo), (ii) sofosbuvir and ledipasvir, (iii) grazoprevir and elbasvir, (iv) sofosbuvir monotherapy, (v) sofosbuvir and daclatasvir, (vi) sofosbuvir and velpatasvir, and (vii) sofosbuvir and simeprevir.

2.2. Measures. Multiple variables were obtained from patients' documentation retrospectively. Variables included (i) demographics, age and gender; (ii) the parameters of glucose metabolism, fasting plasma glucose, set diagnose of T2DM or IFG, and respective treatment; (iii) information about HCV infection, duration, previous treatment, genotype, serum levels of HCV RNA, DAA treatment and duration, and ribavirin; (iv) presence of coinfections, HBs antigen, and antiHIV antibodies; (v) dyslipidemia and arterial hypertension treatment information; (vi) liver fibrosis and function, fibrosis stage determined by transient elastography expressed in kPa, Child-Pugh score in cirrhotics, presence of HCC, and extrahepatic manifestations of HCV infection; (vii) selected results of laboratory tests, total cholesterol, LDL-cholesterol, triglycerides, HDL-cholesterol, creatinine, C-reactive protein, hemoglobin level, neutrophils, platelets, alpha fetoprotein, albumin, INR, AST, ALT, ALP, GMT relative to the ULN, and bilirubin both total and conjugated; and (viii) available variables used to calculate noninvasive fibrosis scores APRI [14], FORNS [15], and FIB-4 [16].

All laboratory tests and virus-related tests were done by standardized, routinely used laboratory methods. Each participating centres performed these tests independently.

The degree of fibrosis was evaluated either histologically or by transient elastography using FibroScan touch 502 device (Echosens, France). Cut-off values used were 9.5 kPa for F3 (Metavir) stage and 12.5 kPa for F4 (Metavir) fibrosis [17]. Decompensation of liver fibrosis was evaluated by calculation of Child-Pugh score, where B or C class was considered a decompensation.

Data were collected at baseline (before treatment start), at the end of treatment (EoT), and 12 weeks after the end of treatment (EoT12w).

Atherogenic index of plasma was calculated as *log (triglycerides/HDL-cholesterol)* [18]

Impaired fasting glucose was defined as glycemia after overnight fasting from 5.6 mmol/L (100 mg/dL) to 6.9 mmol/L (125 mg/dL) and no T2DM history. Type 2 diabetes mellitus was defined as fasting glycemia ≥7.0mmol/L (≥126 mg/dL) or a history of T2DM or antidiabetic treatment [19].

2.3. Statistical Analysis. Data is presented as mean ± standard error of mean or absolute (relative) counts. HCV RNA was analysed after log transformation. First, we compared variables among three categories of fibrosis (Metavir F0-F2 versus F3 versus F4) by using either ANOVA or its nonparametric alternative, Kruskal Wallis for interval and chi-square for categorical variables. Next, we assessed the correlation between fasting glycemia and fibrosis levels (both elastography and noninvasive scores) by Pearson correlation. We also compared baseline fasting glucose and the prevalence of IFG and T2DM between Child-Pugh A and Child-Pugh B/C patients by student *t*-test and chi-squared test, similarly for the comparison between treatment naïve and experienced patients in general and in selected F4 patients. Then we assessed the risk factors for T2DM before treatment by univariate logistic regression. Next, we explored the evolution of glycemia during the DAA treatment (glycemia levels at baseline, EoT, and EoT12w) in the whole cohort and then split by the fibrosis levels, Child-Pugh categories, and treatment experience by Friedman test for three related samples. Finally, we explored the factors that may contribute to the observed decrease of glycemia after treatment by univariate logistic regression.

3. Results

Study cohort consisted of 370 patients altogether; there were several variables with missing data. These patients were omitted from analysis on a case-per-case basis.

Only one patient (0.3%) had F0 fibrosis, 51 patients (13.8%) had F1 fibrosis, 66 patients (17.8) had F2 fibrosis, 80 patients (21.6%) had F3 fibrosis, and 170 patients (45.9%) had F4 fibrosis/cirrhosis. There was statistically significant overrepresentation of F4 patients (p<0.0001). Two patients had missing data on the degree of fibrosis. There was no difference in the proportion of treatment experienced patients between Child-Pugh A versus B/C class (119, 73.5% versus 13, 76.5%; p=0,788).

3.1. Baseline Associations with Glucose Metabolism Disturbances. Baseline, pretreatment parameters are summarized in Tables 1 and 2.

Patients with more advanced fibrosis were older, had higher BMI, and were more commonly treated for dyslipidemia and arterial hypertension. Extrahepatic manifestations and treatment naïve patients occurred mostly in F0-F2 category.

As seen in Table 2, most patients were treated with 3D combo with ribavirin. There was no significant difference in treatment duration between fibrosis categories. Sustained virological response rate was very high. The differences in routine laboratory parameters between fibrosis groups were in line with expectations, except for creatinine, which was the highest in F0-F2 group. Mean fasting glycemia levels were significantly raising with each category of fibrosis. Patients with F4 fibrosis had significantly lower levels of total and LDL cholesterol.

Out of all F4 patients, two patients (1.2%) were classified into Child-Pugh C class, 15 (8.8%) were classified into Child-Pugh B class, and 153 (90%) were in the Child-Pugh A class.

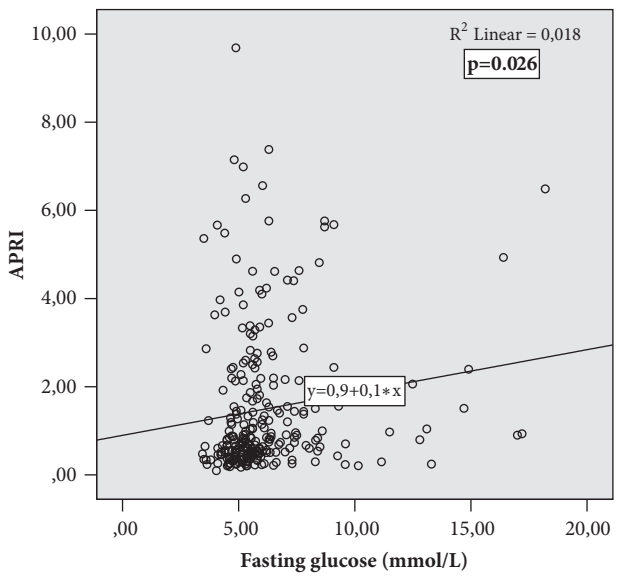

Figure 1: Correlation between fasting glycemia and APRI noninvasive score of fibrosis.

Twenty patients (5.4%) had terminal kidney failure (K/DOQI 5) and were on renal replacement therapy. The majority (78.9%) were F0-F2 fibrosis, 10.5% were F3, and another 10.5% were F4 fibrosis (p<0.0001)

Weak correlations were observed between fasting glycemia and APRI (R^2 =0.018, p=0.026 (Figure 1)), Forns (R^2= 0.04, p=0.001), and FIB-4 scores (R^2 = 0.017. p=0.031). Correlations between fasting glycemia and stiffness by transient elastography (R^2 =0.013, p=0.058) and thrombocytes (R^2 =-0.009, p=0.071) were also almost significant.

The prevalence of IFG or T2DM significantly increased with the degree of liver fibrosis. In F4 Metavir stage, more than half of the patients had IFG or T2DM. Type 2 DM only was present in 14.4% patients with F0-F2 fibrosis, 21.3% patients with F3, and 31.8% with F4 fibrosis. More than half of the patients had either IFG or T2DM in F4 fibrosis category (Table 3). However, the levels of fasting glycemia or prevalence of IFG or T2DM were not different between Child-Pugh A and B/C patients.

Patients with treatment experience had overall higher levels of fasting glycemia and higher prevalence of IFG but not T2DM. This was not the case in the subgroup of F4 fibrosis patients (Table 4).

Table 5 summarizes a series of univariate logistic regression models that were used to assess the predictors of T2DM in chronic hepatitis C. Only age, BMI, and F4 fibrosis were associated with higher risk of T2DM, even after adjustment for each other. Overall multivariate model containing age, BMI, and the presence of F4 fibrosis had a model fit of R^2=0.124.

3.2. Evolution of Glycemia with DAA Treatment. Figures 2, 3, 4, and 5 depict the changes of glycemia in all patients and selected subgroups. In Figure 2 a significant decrease of glycemia, in all but patients without T2DM or IFG, is visible. Figure 3 shows significant decrease of fasting glycemia only

Table 1: Baseline parameters of the study cohort by degrees of fibrosis.

	F0-F2 (Metavir) N=118 Absolute (relative) counts or mean ± SEM	F3 (Metavir) N=80 Absolute (relative) counts or mean ± SEM	F4 (Metavir) N=170 Absolute (relative) counts or mean ± SEM	P
Male sex	58 (49.2)	34 (42.5)	75 (42.4)	0.477
Age (years)	53.64±1.339	60.56±1.297	61.08±0.743	<0.0001
Duration of HCV infection (years)	9.37±0.72	10.82±0.75	11.06±0.59	0.158
BMI	26.17±0.50	27.96±0.51	26.94±0.35	0.037
genotype 1b	93 (78.8)	73 (91.3)	149 (87.6)	0.002
genotype 1a	14 (11.9)	5 (6.3)	4 (2.4)	
genotype 1 (unspecified)	4 (3.4)	2 (2.5)	13 (7.6)	
genotype 3	7 (5.9)	0	4 (2.4)	
genotype other	0	0	0	
Naïve	53 (44.9)	14 (17.5)	43 (25.3)	<0.0001
Experienced	65 (55.1)	66 (82.5)	127 (74.7)	
Relapse	19 (32.2)	35 (56.5)	38 (30.9)	0.009
Partial response	9 (15.3)	7 (11.3)	14 (11.4)	
Breakthrough	5 (8.5)	8 (12.9)	14 (11.4)	
Nonresponse	26 (44.1)	12 (19.4)	57 (56.3)	
HBsAg positive	3 (2.5)	0	1 (0.6)	0.166
HIV positive	0	0	0	N/A
Treatment for hypertension	33 (30.3)	35 (46.7)	72 (47.1)	0.015
Treatment for dyslipidemia	7 (6.4)	11 (14.7)	41 (26.8)	<0.0001
Treatment for T2DM	12 (10.2)	12 (15.0)	34 (20.0)	0.078
Diet only	2 (20)	5 (55.6)	8 (30.8)	0.024
OAD only	5 (50)	4 (44.4)	4 (15.4)	
Insulin	3 (30)	0	14 (53.8)	
Extrahepatic manifestations	23 (19.5)	10 (12.5)	15 (8.8)	0.03
HCC	1 (0.8)	2 (2.5)	5 (2.9)	0.475

in treatment experienced patients. Ultimately, Figure 4 shows that in F4 patients the decrease of fasting glycemia happened only in Child-Pugh A patients.

Thirty T2DM patients were on antidiabetic medication before the start of DAA therapy, 13 were taking oral antidiabetics, and 17 were taking insulin. None of the patients treated with oral antidiabetics had a reduction in the dosage of antidiabetics during the DAA treatment or in 12-week follow-up. Three insulin treated patients needed an insulin dose reduction due to documented hypoglycemia (10% of all treated patients and 17.6% of insulin treated patients).

Finally, we were interested in the predictors of significant (>5%) decrease of glycemia after treatment, which happened in 47.8% of patients. Serial univariate logistic regression showed that only female sex and baseline glycemia predicted the achievement of significant glycemia decrease in the whole cohort. Degrees of fibrosis or treatment experience were not predictors of posttreatment glycemia decrease (Table 6). Baseline glycemia was a significant predictor of fasting glycemia decrease after treatment event after adjustment for age and sex (OR 1.498; 95% CI 1.210-1.854).

4. Discussion

This study analysed retrospective data of Slovak patients before the start of DAA treatment for hepatitis C.

Baseline fasting glycemia was increasing with the degree of liver fibrosis. We observed weak correlation between fasting glycemia and APRI score (Figure 1), FIB-4, and Forns index; however, the correlation between fasting glycemia and liver stiffness by transient elastography did not reach statistical significance, probably due to the small sample size. These conflicting results are probably due to low number of analysed patients.

Our results corroborate previously published data that show high prevalence of T2DM in patients with hepatitis C, with prevalence increasing with the degree of fibrosis [9, 11]. Patients with cirrhosis due to hepatitis C have significantly higher prevalence of T2DM compared to different etiologies of cirrhosis [11]

Treatment experienced patients had significantly higher baseline glycemia, higher prevalence of IFG, and higher prevalence of either IFG or T2DM compared to treatment

Table 2: Baseline laboratory parameters and DAA treatment parameters by degree of fibrosis.

	F0-F2 N=118 Absolute (relative) counts or mean ± SEM	F3 N=80 Absolute (relative) counts or mean ± SEM	F4 N=170 Absolute (relative) counts or mean ± SEM	P
HCV RNA IU/mL	2 945 961±640 644	2 428 590±429 297	2 799 155±348 275	0.427 K-W test
Treatment				
3D combo	83 (70.3)	43 (53.8)	96 (57.8)	<0.0001
SOF LDV	21 (17.8)	35 (43.8)	64 (38.6)	
other	14 (11.9)	2 (2.5)	6 (3.6)	
Ribavirin	19 (16.2)	18 (22.8)	106 (63.9)	<0.0001
Treatment duration				
<8 weeks (incomplete)	4 (3.4)	1 (1.3)	4 (2.4)	0.611
8 weeks	2 (1.7)	0	0	
12 weeks	109 (93.2)	76 (95)	155 (93.4)	
24 weeks	2 (1.7)	3 (3.8)	7 (4.2)	
SVR	89 (96.7)	74 (97.4)	144 (96.0)	0.862
Creatinine umol/L (n=366)	139.5±17.5	84.2±6.2	79.9±5.8	<0.0001
CRP mg/dL (n=127)	3.5±0.78	4.3±1.60	1.6±0.19	0.012
Hb g/L (n=368)	142±1.7	144±2	138±1.4	0.023
Neutrophils 10^9/L (n=258)	4.01±0.18	3.32±0.17	2.8±013	<0.0001
Platelets 10^9/L (n=368)	206±6.9	200±7.5	132±5	<0.0001
AFP kIU/L (n=240)	5.4±0.9	7.6±0.6	22.3±0.2	<0.0001
Albumin g/L (n=354)	41.6±0.4	41.5±0.4	38.6±0.4	<0.0001
INR (n=335)	1.02±0.02	1.05±0.03	1.13±0.01	<0.0001
AST %ULN (n=281)	133.6±14.5	145.6±10.0	234.3±14.9	<0.0001
ALT %ULN (n=368)	175.40±14.2	166.2±10.8	229.5±13.9	0.002
GMT %ULN (n=281)	154.6±15.6	144.3±14.4	241.5±30.4	0.01
ALP %ULN (n=281)	76.7±4.1	69.1±2.9	92.9±4.1	<0.0001
Bilirubin umol/L (n=368)	12.9±1.0	13.2±0.5	19.7±1.0	<0.0001
Conj. Bil. umol/L (n=184)	5.4±1.6	3.6±0.3	8.5±1.03	0.01
Glucose (mmol/l)	5.75±0.18	5.84±0.17	6.69±0.2	0.001
Total cholesterol (mmol/L) n=328	4.45±0.10	4.69±0.10	4.19±0.08	0.001
HDL-C (mmol/L) n=140	1.34±0.05	1.32±0.07	1.27±0.06	0.622
LDL-C (mmol/L) n=136	2.73±0.12	2.48±0.15	2.33±0.09	0.02
Triglycerides (mmol/L) n=201	1.37±0.08	1.27±0.10	1.54±0.09	0.165
AIP n=140	-0.03±0.03	-0.03±0.05	0.04±0.04	0.272
FORNS	5.74±0.23	6.18±0.23	8.19±0.18	<0.0001
FIB-4	1.8±0.15	2.55±0.27	5.77±0.54	<0.0001
APRI	0.76±0.09	0.9±0.1	2.27±0.18	<0.0001
TELP (kPa)	6.14±0.23	7.61±0.47	20.07±1.27	<0.0001

Table 3: Prevalence of impaired fasting glucose and type 2 DM by the degree of fibrosis.

	F0-F2 N=118 Absolute (relative) counts	F3 N=80 Absolute (relative) counts	F4 N=170 Absolute (relative) counts	P
IFG	27 (22.8)	19 (23.8)	46 (27.1)	0.157
T2DM	17 (14.4)	17 (21.3)	54 (31.8)	0.004
IFG or T2DM	44 (37.3)	36 (45.0)	100 (58.8)	0.001

Table 4: Baseline levels of fasting glycemia and impaired fasting glucose or type 2 diabetes mellitus prevalence according to treatment experience.

	Naïve Absolute (relative) counts or mean±SEM	Treatment experienced Absolute (relative) counts or mean±SEM	P
All patients			
Glycemia (mmol/L)	5.71±0.15	6.41±0.16	0.006
IFG	19 (17.1)	74 (28.6)	0.008
T2DM	23 (20.7)	65 (25.1)	0.411
IFG or T2DM	42 (37.8)	139 (53.7)	0.005
Only F4 fibrosis patients			
Glycemia (mmol/L)	6.16±0.23	6.86±0.26	0.637
IFG	10 (23.3)	36 (28.3)	0.535
T2DM	13 (30.2)	41 (32.3)	0.921
IFG or T2DM	23 (53.5)	77 (60.6)	0.411

Table 5: Association of type 2 diabetes mellitus with various predictors.

	No T2DM (mean±SEM or Absolute (relative) counts	T2DM (mean±SEM or Absolute (relative) counts	P	OR (univariate logistic regression)	95%CI
Age (years)	58±1	63±1	<0.0001	1.04	1.02-1.07
Male sex	115 (42.6)	45 (51.1)	0.162	1.41	0.87-2.28
BMI (kg/m^2)	26.6±0.27	28.5±0.6	0.001	1.099	1.035-1.167
HCV RNA (IU/mL)	2 776 629±347 176	2 937 405±434 013	0.259	1.225	0.862-1.741
Treatment experienced	187 (69.3)	65 (73.9)	0.411	1.254	0.73-2.16
Elastography (kPa)	12.5±0.86	13.6±1.3	0.5	1.007	0.99-1.03
F4 fibrosis (elastography)	113 (42.2)	54 (61.4)	0.004	2.67	1.45-4.91
F3 fibrosis (elastography)	60 (22.4)	17 (19.3)	0.227	1.58	0.75-3.34
Duration of HCV infection	10.44±0.44	10.56±0.85	0.900	1.002	0.97-1.047

Table 6: Predictors of significant (>5%) decrease of glycemia after DAA treatment.

	Glycemia 5% decrease not achieved (mean±SEM or absolute (relative) counts	Glycemia 5% decrease achieved (mean±SEM or absolute (relative) counts	P	OR	95%CI
Age (years)	60±1	61±1	0.303	1.01	0.97-1.04
Male sex	43 (45.3)	25 (30.5)	0.044	0.53	0.26-0.99
BMI (kg/m^2)	26.9±0.46	27.2±0.44	0.807	1.015	0.93-1.10
HCV RNA (IU/mL)	2 380 969±443 997	3 404 197±595 764	0.092	1.369	0.91-2.06
Treatment experienced	72 (75.8)	66 (80.5)	0.452	1.32	0.64-2.71
Elastography (kPa)	9.6±1.23	11.1±1.41	0.849	1.01	0.98-1.04
F4 fibrosis (elastography)	45 (47.4)	44 (55.0)	0.363	1.43	0.66-3.12
F3 fibrosis (elastography)	28 (29.5)	21 (26.3)	0.829	1.10	0.46-2.62
Thrombocytes x10^9/L	172±7.7	157±9.5	0.07	0.997	0.994-1.001
APRI	1.43±0.16	1.56±0.16	0.558	1.063	0.87-1.31
Baseline fasting glucose (mmol/L)	5.7±1.8	7.2±2.7	<0.0001	1.431	1.177-1.740

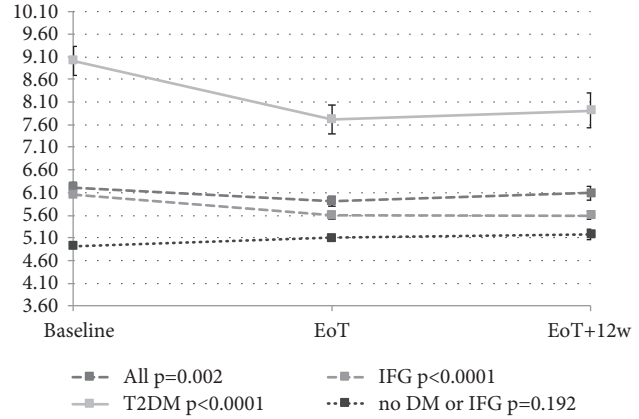

Figure 2: Changes of fasting glycemia with treatment in all patients and subgroups of patients with type 2 diabetes mellitus or impaired fasting glucose. EoT: end of treatment; EoT+12w: 12 weeks after the end of treatment.

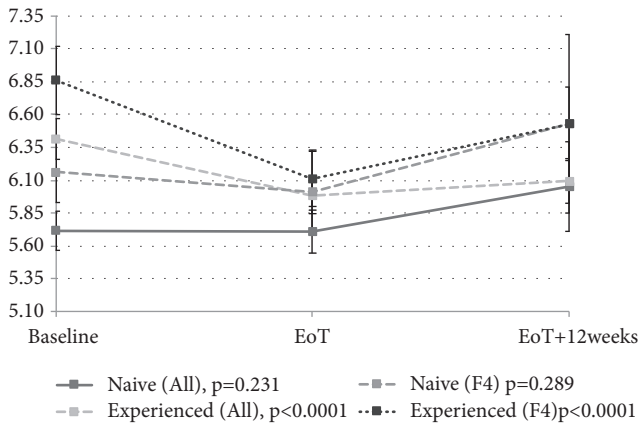

Figure 3: Changes of fasting glycemia with treatment in naive and experienced patients in the whole group and separately for F4 fibrosis patients. EoT: end of treatment; EoT+12w: 12 weeks after the end of treatment.

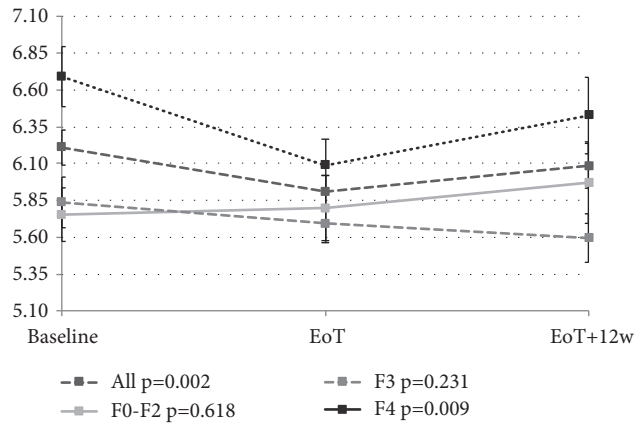

Figure 4: Changes of fasting glycemia with treatment in the whole group and separately for F0-F2, F3, and F4 (Metavir) fibrosis patients. EoT: end of treatment; EoT+12w: 12 weeks after the end of treatment.

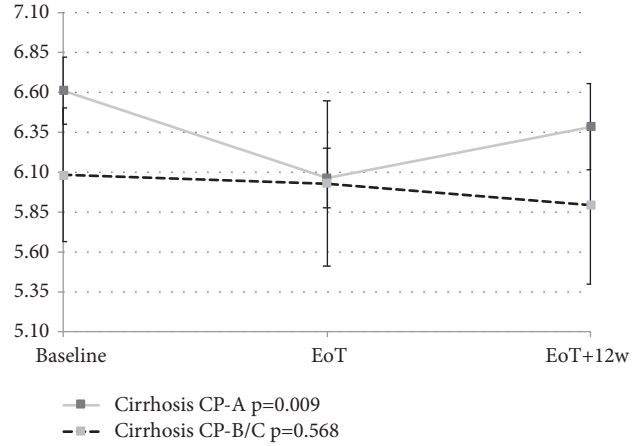

Figure 5: Changes of fasting glycemia with treatment in patients with Child-Pugh A and Child-Pugh B/C cirrhosis. EoT: end of treatment; EoT+12w: 12 weeks after the end of treatment.

naive patients. It has been shown that insulin resistance is associated with worse outcome of interferon-based treatment [20] and that insulin resistance is associated with higher degree of fibrosis [8]. The highest number of treatment experienced patients was among cirrhotics also in our cohort. Therefore, we expected also higher proportion of T2DM patients in treatment experienced group. When we considered only F4 patients there was no difference between the prevalence of T2DM or IFG between experienced and naive patients or patients with compensated and decompensated cirrhosis.

In our study, only predictive factors for baseline T2DM were higher age, higher BMI, and F4 fibrosis. No association was found between HCV RNA levels, the duration of HCV infection, gender or previous treatment, and T2DM. There is a discrepancy between these results and published data, where the risk of T2DM was associated also with the duration of HCV infection and the response to previous treatment, together with family history of T2DM and insulin sensitivity [1].

Treatment was, as expected, highly effective, with more than 95% achieving sustained virological response, even in F4 fibrosis or Child-Pugh B/C cirrhosis groups. The achievement of SVR may not be the only benefit of DAA treatment. The eradication of the HCV has positive effect also on the extrahepatic manifestations of chronic HCV infection. Three studies have already shown that the achievement of SVR is associated with the decreased risk of future T2DM [9, 21, 22]. In a retrospective study Arase et al. reported that chronic HCV patients that do not respond to interferon-based therapy have almost threefold risk of future T2DM even after adjustment for age, cirrhosis, and prediabetes before the start of the treatment [9]. The achievement of SVR reduced the risk of future T2DM more than twofold in a Spanish prospective study [21]. Similar results were reported in a retrospective analysis from Barcelona [22]. This decrease of T2DM risk was not described in an Italian study with more than 8-year follow-up; however, this study included only small number of patients [23].

4.1. Treatment Effect. In this study we also evaluated the dynamics of glycemia before treatment, at EoT, and 12 weeks after EoT in general and in subgroups based on the degree of fibrosis, compensation of cirrhosis, and treatment experience.

Antiviral treatment of chronic hepatitis C may lead to the improvement of glucose metabolism mainly in patients who achieved SVR. During 48-week interferon-based treatment a significant decrease of fasting glucose and glycated hemoglobin (HbA1c) was observed in patients who achieved SVR but not in relapsers [24]. Our study evaluated the changes in fasting glycemia after the EoT and 12 weeks after EoT. We observed significant decrease of fasting glycemia in all patients ($p=0.002$). This decrease was the highest in diabetics and patients with IFG ($p<0.0001$ for both). However, glycemia did not change in patients without IFG or T2DM. These results confirm the findings of post hoc analysis of 6 registration phase 3a studies for paritaprevir/ritonavir + dasabuvir + ombitasvir. Treated patients had significant decrease of fasting glycemia compared to placebo patients. This decrease was observed particularly in patients with T2DM and patients with prediabetes. Patients without prediabetes or T2DM experienced a slight increase of fasting glycemia [25].

More studies documented the decrease of fasting glucose levels and HbA1c [26–29], fasting glucose [30], or HbA1c [31, 32] in patients with chronic hepatitis C and T2DM either during the treatment period or at the time of SVR. Significant decrease of fasting glucose occurs in the first four weeks of DAA treatment [25, 30] and persists after the treatment ending. Significant decrease of HbA1c was also observed in patients with chronic hepatitis C treated by DAA after liver transplantation [33]. Only one prospective cohort study failed to show a significant decrease of fasting glycemia nor HbA1c in patients with and without HIV coinfection regardless of the presence of T2DM [34]. Glycemia improvement is not observed after DAA treatment in all patients with chronic hepatitis C. Italian authors documented the decrease of fasting glycemia in 67% and HbA1c in 80% of patients treated with DAA [26]. Egyptian authors observed the improvement of glycemic control in 77.2% of genotype 4 chronic hepatitis C and T2DM patients who achieved SVR twelve weeks after the treatment conclusion [27].

One of the side effects of DAA treatment of chronic hepatitis C in T2DM patient may be hypoglycemia. Spanish authors described a case of symptomatic hypoglycemia in T2DM hepatitis C patient at 18th day of sofosbuvir/ledipasvir treatment despite radical decrease of insulin dosage [35]. Indeed, the reduction of antidiabetic treatment is required in 8%-40% of these patients, particularly in those treated with insulin [26, 27, 29, 31–33]. In our cohort of patient, the reduction of antidiabetic medication occurred in 10% of patients, all of them treated with insulin.

We observed significant decrease of glycemia in F4 fibrosis patients, but not in F3 or F0-F2 fibrosis patients. This is probably due to higher prevalence of T2DM or IFG in F4 fibrosis patients. Moreover, significant decrease of glycemia was observed only in Child-Pugh A, but not B or C cirrhotic patients. Similarly, in the Egyptian study the glycemic control was improved more commonly in Child-Pugh A patients. Since we did not observe any difference between Child-Pugh A and B/C patients in baseline glycemia, prevalence of T2DM or IFG, or treatment experience we suspect it is related to lower liver glycogen stores in decompensated cirrhosis.

When we considered treatment experience, we observed that significant decrease of glycemia occurred only in treatment experienced patients compared to treatment naive patients. Treatment experienced patient had significantly higher baseline fasting glycemia, significantly higher prevalence of IFG or T2DM, and significantly higher rate of F4 fibrosis. Similarly, significant decrease of glycemia occurred only in treatment experienced cirrhotics compared to naive.

Because of very few patients who sustained treatment failure, we could not compare the dynamics of glycemia between patients who did and did not achieve a SVR. In a study from US, patients without cirrhosis who achieved SVR had significantly higher reduction of HbA1c compared to patients who failed the treatment [31].

Insulin resistance and T2DM play the principal role in the pathophysiology of atherosclerosis. Patients with chronic hepatitis C have elevated cardiovascular risk. The achievement of SVR in nondiabetic patients may reduce future T2DM prevalence in this cohort. The achievement of SVR in diabetic patients is associated with improved glycemic control. Thus one could logically extrapolate that the eradication of HCV infection through DAA treatment may indeed lead to the decrease of cardiovascular risk [36].

4.2. Limitations. This study has several limitations; probably the greatest is its retrospective design and thus the omission of more important data, e.g., the data on HbA1c before, during, and after DAA treatment. Another limitation is a relatively lower number of participants, out of which only minority had T2DM or IFG. The data is limited also by short time of follow-up after the finish of DAA treatment. Finally, patients received different DAA medications. A prospective multicentric study with larger cohort of patients may be able to explain the changes in the insulin resistance in hepatitis C patient more thoroughly. Such study will be very difficult to undertake, since large proportion of treatment experienced and cirrhotic hepatitis C patients in Europe underwent successful treatment already [37].

5. Conclusions

This retrospective study confirmed that the prevalence of either T2DM or IFG increases in chronic hepatitis C patients with the degree of fibrosis; patients with F4 fibrosis had 27.1% prevalence of IFG and 31.8% of T2DM. The predictive factors for T2DM had besides F4 fibrosis also higher age and BMI. Significant decrease of fasting glycemia at the end of treatment and 12 weeks after that was observed in the whole cohort and in subgroups of patients with type 2 diabetes mellitus, impaired fasting glucose, Child-Pugh A cirrhotic patients, treatment experienced patients, and treatment experienced cirrhotics. Long term follow-up may further show if the achievement of SVR after DAA treatment will reduce the risk of future T2DM development similarly

to SVR after interferon treatment and if the improvement of glycemic control in patients with T2DM decreases the risk of chronic complications and improves survival.

Data Availability

The data used to support the findings of this study cannot be made available in order to protect patient privacy.

Conflicts of Interest

Sylvia Drazilova reports personal fees and nonfinancial support from Abbvie, Gilead, and MSD, outside the submitted work. Martin Janicko reports personal fees and nonfinancial support from AbbVie and nonfinancial support from Gilead, outside the submitted work. Lubomir Skladany reports grants, personal fees, and nonfinancial support from Abbvie, Gilead, and MSD, outside the submitted work. Pavol Kristian reports personal fees and nonfinancial support from AbbVie and Gilead and personal fees from MSD, outside the submitted work. Marian Oltman reports personal fees and nonfinancial support from Abbvie, Gilead, and MSD, outside the submitted work. Maria Szantova, reports grants, personal fees, and nonfinancial support from Gilead, personal fees and nonfinancial support from MSD, and nonfinancial support from Abbvie, outside the submitted work. Dusan Krkoska reports nonfinancial support from AbbVie and Gilead, outside the submitted work. Eva Mazuchova reports nonfinancial support from AbbVie, Gilead, and MSD, outside the submitted work. Lubica Piesecka reports personal fees and nonfinancial support from Abbvie, Gilead, and MSD, outside the submitted work. Veronika Vahalova reports personal fees and nonfinancial support from Abbvie, Gilead, and MSD, outside the submitted work. Marek Rac reports personal fees and nonfinancial support from Abbvie, Gilead, and MSD and nonfinancial support from Jannsen, outside the submitted work. Ivan Schreter reports personal fees and nonfinancial support from AbbVie, Gilead, and Jannsen and personal fees from MSD, outside the submitted work. Ladislav Virag reports nonpersonal fees and nonfinancial support from AbbVie and MSD and financial support from Gilead, outside the submitted work. Tomas Koller reports personal fees and nonfinancial support AbbVie, personal fee from MSD, and nonfinancial support from Gilead, outside the submitted work. Adriana Liptakova reports contract with AbbVie. Miriam Ondrasova reports contract with AbbVie. Peter Jarcuska reports personal fees and nonfinancial support from AbbVie and Gilead and personal fees from MSD, outside the submitted work.

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Research Article

Characteristics and Changes over Time of Alcohol-Related Chronic Liver Diseases in Italy

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Introduction. To evaluate the characteristics of alcohol-related chronic liver disease (CLD) in Italy and their potential changes over time. *Patients and Methods*. Subjects with CLD were enrolled in two national surveys performed in 2001 and in 2014 in Italy. The two surveys prospectively recruited patients aged ≥ 18 years referring to more than 80 Italian liver units scattered all over the country using similar clinical approach, analytical methods, and threshold of risky alcohol intake definition (≥ 3 units/day in men and ≥ 2 units/day in women). *Results*. Out of 12,256 enrolled subjects, 2,717 (22.2%) reported a risky alcohol intake. Of them, anti-HCV positive was observed in 48.3% of subjects. The overall sex ratio (M/F) was 3.1, decreasing from 3.8 in 2001 to 1.3 in 2014. Women were significantly older than men (58.9 versus 53.1 years; $p < 0.01$) and an increasing ageing over time was observed in both sexes. The proportion of subjects with liver cirrhosis increased over time in both sexes, and decompensated stage (Child B or C) was detected in 55.9% of cases in 2001 and 46.0% in 2014. *Conclusions*. Risky alcohol intake plays a role in more than one-fifth of CLD in Italy, with a shift over time towards an older age and a more severe liver disease stage. These data put alcohol back in the spotlight with an important role in CLD in the years to come in Italy.

1. Introduction

Alcohol consumption is a major cause of chronic liver disease, particularly in Western and Eastern Europe, where the highest worldwide per capita intake of alcohol is reported [1, 2]. In the United Kingdom there was a 5-fold increase in cirrhosis mortality among men and a 4-fold increase among women from 1950 through 2000, which parallels the doubled alcohol consumption recorded in these countries over the same period [3]. However, only about 35% of heavy drinkers develop the disease [4], suggesting that, beyond alcohol abuse, other factors are involved in the development of liver cirrhosis [5]. In Italy, a significant decrease in the proportion of risky alcohol consumers has been over time observed, from 21.3% in 2007 to 15.7% in 2015. This downtrend was greater in men (from 30.6% to 23.0%) than in women (from 12.6% to 9.0%) [6].

The wealth on information on hepatitis viruses has decreased the attention paid to the role of alcohol intake in the development of chronic liver disease (CLD) and progression

to cirrhosis, so that information on alcoholic liver diseases in Italy is scanty and fragmentary.

Pooling the cases recruited in two Italian national surveys performed in 2001 [7] and in 2014 [8, 9] on the characteristics of subjects with chronic liver diseases (CLD) referring more than 80 liver units, we have evaluated the main aspects of alcohol-related CLD and their potential changes over time.

2. Patients and Methods

The two national surveys have been previously described [7–9]. The first one enrolled 9,997 persons with CLD consecutively referring to 79 hospital liver units for a six months' period in 2001. The second one recruited 2,557 CLD cases consecutively referring to 15 hospital liver units in 2014. Subjects enrolled in 2014 were different from those in the 2001 survey. Criteria of enrolment were aged over 18 years and either altered hepatic biochemistry, presence of etiologic markers of liver damage, or history of symptoms consistent with CLD. Both inpatients and outpatients were recruited. In both studies, the enrolling liver units were scattered all over the country. Most of the 15 hospitals participating in the second study had also taken part in the first one. The liver units participating to the two surveys collected the data prospectively had comparable access procedures and used similar approach and similar analytical methods.

Personal data were collected in full compliance with the Italian law on personal data protection, and each patient gave his/her informed consent to participate. All procedures applied in the two studies were in accordance with the international guidelines, with the standards of human experimentation of the local Ethics Committees and with the Helsinki Declaration of 1975, revised in 1983. At the time of the first observation, each patient signed their informed consent for the collection of personal data. Patients who agreed to undergo liver biopsy signed an appropriate informed consent before biopsy was performed. Patients were enrolled only once at their first observation. For each patient, a precoded questionnaire containing demographic, epidemiological, and clinical data was filled out. No patient refused to participate in the studies.

2.1. Diagnostic Criteria. In both surveys, the presence of serum hepatitis B surface antigen (HBsAg) identified hepatitis B virus (HBV) aetiology and that of antibody to hepatitis C virus (anti-HCV), an HCV aetiology. Autoimmune chronic hepatitis and primary biliary cholangitis, hereditary hemochromatosis, and Wilson's disease were diagnosed according to standardized international criteria [10–13].

An alcohol intake > 40 g/day for males (≥ 3 drinks a day) and > 30 g/day for females (≥ 2 drinks a day) for at least 5 years was considered an etiologic factor for alcoholic liver disease [14, 15].

Nonalcoholic fatty liver disease (NAFLD/NASH) was diagnosed based on abnormal serum values of alanine aminotransferase (ALT) associated with hepatic steatosis identified by liver histology and/or ultrasound (US), in the absence of other known causes of liver disease [16]. CLD was considered cryptogenic in the absence of any viral, autoimmune, or metabolic aetiology.

Chronic hepatitis was diagnosed based on liver histology, when available, or on the persistence (>6 months) of abnormal ALT in the absence of clinical, biochemical, and US evidence of liver cirrhosis [17]. Liver cirrhosis was diagnosed by liver biopsy (LB) or, in the absence, on the presence of characteristic clinical, biochemical, and ultrasound signs [17]. The diagnosis of hepatocellular carcinoma (HCC) was based on histological and/or imaging findings and alfa-1-fetoprotein serum levels [18].

Percutaneous LB was performed, if requested by the physician in care for diagnostic purposes, under US guidance using a disposable modified Menghini's needle. In each liver unit, a skilled pathologist unaware of the clinical and laboratory data evaluated liver histology. Liver necro-inflammation and fibrosis were assessed by the Ishak [19] or Metavir scoring system [20] and standardized criteria were used to convert the Ishak score to a Metavir scores [21]. Liver biopsy slices were read by local of central pathologist.

2.2. Serologic Assays. Serum HBsAg and antibody to HCV were sought using commercial immunoenzymatic assays. Routine tests were applied to seek the etiologic markers of autoimmune hepatitis, primary biliary cholangitis, iron and copper overload, and liver functions.

2.3. Statistical Analysis. The data were collected in a preestablished electronic CRF database (web-based data collection, e-CRF provided by Air-Tel®, Airon Telematica, Milan, Italy). Differences in means and in proportion were evaluated by Student's t-test and chi square test or Fisher's exact test, respectively. A p value < 0.05 was considered to be significant. All p values were two-tailed.

3. Results

Out of the 12,754 enrolled subjects with CLD (9,997 in 2001 and 2,557 in 2014), 2,717 (22.2%) reported a risky alcohol intake. The mean age was 54.2 years with a sex ratio (M/F) of 3.2. The mean Body Mass Index (BMI) resulted in 25.6. A nearly similar proportion of cases was observed in northern/central areas (47.7%) and in southern/islands (49.8%). Nearly one-third (35.5%) of subjects had liver cirrhosis without (30.7%) or with (4.5%) HCC. Of these 2,717 patients, 1,313 (48.3%) were also anti-HCV positive, while 1,163 (42.7 %) reported a risky alcohol intake as the only etiologic agent of CLD. The comparison of the two studies shows an over time statistically significant older mean age (53.2 years versus 60.2 years, $p < 0.001$), a decreasing M/F sex ratio (from 3.8 to 1.3, $p < 0.001$), an increasing proportion of cases from northern/central areas ($p < 0.001$), an increase in the proportion of subjects declaring alcohol abuse with a decrease in the proportion of those having alcoholism plus anti-HCV positivity ($p < 0.02$), and, finally, an increase of subjects with liver cirrhosis without or with HCC (39.9% versus 43.4%, $p < 0.01$) (Table 1).

Table 1: Baseline characteristics of 2,717 enrolled subjects with chronic liver disease alcohol related in Italy, by year of recruitment.

Characteristic	Overall (n=2,717)	Study 2001 (n=2,342)	Study 2014 (n=375)	p-value
Age, years, mean ± SD	54.2 ± 12.8	53.2 ± 14.8	60.2 ± 13.4	<0.001
Sex Ratio (M/F)	3.1	3.8	1.3	<0.001
BMI, mean ± SD	25.6 ± 3.8	25.5 ± 3.8	23.0 ± 4.3	0.015
Area of birth				
(i) Northern/Central Italy	1,295 (47.7%)	1,099 (46.9%)	196 (52.3%)	<0.001
(ii) Southern Italy/Islands	1,354 (49.8%)	1,192 (50.9%)	159 (42.4%)	
(iii) Born abroad	71 (2.6%)	51 (2.2%)	20 (5.3%)	
Risk factors				
(i) Alcohol intake alone	1,163 (42.8%)	983 (42.0%)	179 (47.7%)	0.02
(ii) Alcohol intake/HBsAg positive	172 (6.3%)	140 (6.0%)	32 (8.5%)	
(iii) Alcohol intake/anti-HCV positivity	1,313 (48.3%)	1,157 (49.4%)	154 (41.1%)	
(iv) Alcohol intake/HBsAg positive/anti-HCV positive	72 (6.3%)	62 (2.6%)	10 (2.7%)	
Diagnosis				
(i) Chronic hepatitis	1,762 (64.8%)	1,550 (66.1%)	212 (56.5%)	<0.001
(ii) Liver cirrhosis without HCC	834 (30.7%)	697 (29.8%)	137 (36.5%)	
(iii) Liver cirrhosis with HCC	121 (4.5%)	95 (4.1%)	26 (6.9%)	

BMI: body mass index. HCC: hepatocellular carcinoma.

Table 2: Characteristics of 2,717 enrolled subjects with chronic liver disease alcohol related in Italy, by sex.

Characteristics	Males (n=2,059)	Females (n=658)	Sex Ratio (M/F)	p-value
Age, years, mean ± SD	53.0 ± 14.7	58.0 ± 14.5	-	<0.001
BMI, mean ± SD	25.7 ± 3.7	25.1 ± 4.4	-	0.001
Area of birth				
(i) Northern/Central Italy	943 (45.8%)	352 (53.5%)	2.7	<0.001
(ii) Southern Italy/Islands	1,069 (51.9%)	282 (42.9%)	3.8	
(iii) Born abroad	47 (2.3%)	24 (3.6%)	2.0	
Risk factors				
(i) Alcohol intake alone	810 (39.3%)	352 (53.5%)	2.3	<0.001
(ii) Alcohol intake/HBsAg positive	147 (7.1%)	25 (3.8%)	5.9	
(iii) Alcohol intake/anti-HCV positivity	1,040 (50.5%)	271 (41.2%)	3.8	
(iv) Alcohol intake/HBsAg positive/anti-HCV positive	62 (3.0%)	10 (1.5%)	6.2	
Diagnosis				
(i) Chronic hepatitis	1,348 (65.5%)	414 (62.9%)	3.3	0.15
(ii) Liver cirrhosis without HCC	614 (29.8%)	220 (33.4%)	2.8	
(iii) Liver cirrhosis with HCC	97 (4.7%)	24 (3.6%)	4.0	

BMI: body mass index. HCC: hepatocellular carcinoma.

As compared to males, females were over time more likely older (58.0 years versus 53.0 years, $p < 0.01$), were born in northern/central areas (53.5% versus 45.7%, $p < 0.01$), and more frequently declared alcohol abuse (53.5% versus 39.3%, $p < 0.01$). No significant difference by gender was observed according to diagnosis (Table 2).

Statistically significant intragender over time differences were observed in males, with an ageing age, an increasing proportion of cases related to alcohol abuse alone, and an increasing proportion of cirrhotic patients with or without HCC (Table 3). Intragender differences over time were observed even in females with an ageing age, a higher proportion of cases with liver cirrhosis, and a decreasing proportion of subjects with alcohol abuse alone (Table 4).

Characteristics of liver cirrhosis cases are reported in Table 5. The most relevant findings are the over time decrease of both the M/F sex ratio ($p < 0.001$) and the rate of cases (55.9% to 46.8%; $p < 0.05$) with in a decompensated stage (Child B/C).

4. Discussion

The present work is a careful subanalysis of data only generically stated in an earlier publication [8], focusing

Table 3: Characteristics of chronic liver diseases alcohol related in male sex over time in Italy.

Characteristics	2001 Study (n°=1,852)	2014 Study (n°=207)	p-value
Age (years), mean ± SD	52.4 ± 14.7	58.0 ± 13.9	<0.001
BMI (kg/m^2)**,** mean ± SD	25.6 ± 3.6	26.4 ± 4.1	<0.05
Area of origin			
(i) Northern/Central Italy	888 (45.8%)	95 (45.9%)	0.03
(ii) Southern Italy/Islands	967 (52.2%)	102 (49.3%)	
(iii) Born abroad	37 (2.0%)	10 (4.8%)	
Risk factors			
(i) Alcohol intake alone	710 (38.3%)	100 (48.3%)	0.015
(ii) Alcohol intake/HBsAg positive	129 (7.0%)	18 (8.7%)	
(iii) Alcohol intake/anti-HCV positivity	957 (51.7%)	83 (40.1%)	
(iv) Alcohol intake/HBsAg positive/anti-HCV positive	56 (3.0%)	6 (2.9%)	
Diagnosis			
(i) Chronic hepatitis	1,231 (66.5%)	117 (56.5%)	0.004
(ii) Liver cirrhosis without HCC	541 (29.2%)	73 (35.3%)	
(iii) Liver cirrhosis with HCC	80 (4.3%)	17 (8.2%)	

BMI: body mass index. HCC: hepatocellular carcinoma.

Table 4: Characteristics of chronic liver diseases alcohol related in female sex over time, in Italy.

Characteristics	2001 Study (n°=490)	2014 Study (n°=168)	p-value
Age (years), mean ± SD	56.3 ± 14.8	63.0 ± 12.3	<0.001
BMI (kg/m^2)**,** mean ± SD	24.9 ± 4.3	25.6 ± 4.4	0.1
Area of origin			
(i) Northern/Central Italy	251 (51.2%)	101 (60.1%)	0.009
(ii) Southern Italy/Islands	225 (45.9%)	57 (33.9%)	
(iii) Born abroad	14 (2.9%)	10 (6.0%)	
Risk factors			
(i) Alcohol intake alone	273 (55.7%)	79 (47.0%)	0.002
(ii) Alcohol intake/HBsAg positive	11 (2.2%)	14 (8.3%)	
(iii) Alcohol intake/anti-HCV positivity	200 (40.8%)	71 (42.3%)	
(iv) Alcohol intake/HBsAg positive/anti-HCV positive	6 (1.2%)	4 (2.4%)	
Diagnosis			
(i) Chronic hepatitis	319 (65.1%)	95 (56.5%)	0.09
(ii) Liver cirrhosis without HCC	156 (31.8%)	64 (38.1%)	
(iii) Liver cirrhosis with HCC	15 (3.1%)	9 (5.4%)	

BMI: body mass index. HCC: hepatocellular carcinoma.

exclusively on alcohol-related CLD. The 2001 and the 2014 nationwide prevalence surveys were structurally similar. Both studies were cross-sectional and prospectively enrolled for a given time inpatients and outpatients aged 18 or more with CLD of any aetiology referring for altered hepatic biochemistry or positivity of hepatitis virus serum markers to one of the participating liver units located all over the country. The same clinical approach, analytical methods, and facilities to access the participating liver units, operating in district general or academic hospitals, have been adopted. In addition, several of these liver units have participated to both 2001 and 2014 surveys. Finally, the same threshold of a risky alcohol intake was adopted in both surveys [14, 15]. Consequently, the pooling and the comparison of the two studies may be no matter of concern. The most striking findings of this large series of subjects with CLD alcohol-related are a male preponderance, an older mean age of women, an increasing ageing over time in both sexes, a high proportion of subjects with a history of alcohol abuse and anti-HCV positive, and the majority of liver cirrhosis cases presenting at a decompensated stage.

The greater vulnerability of women and lower safe limits for alcohol consumption have been long recognized [22]. Women have a higher risk of alcoholic cirrhosis compared to men for a given level of alcohol intake [22, 23]. Some factors may explain this sex difference: lower production

Table 5: Characteristics of 955 subjects with alcohol-related liver cirrhosis in Italy, by year of recruitment.

Characteristics	Overall (n°=955)	Study 2001 (n°=792)	Study 2014 (n°=163)	p-value
Age (years), mean ± SD	60.0 ± 12.3	59.8 ± 12.1	60.7 ± 13.4	0.4
Sex Ratio (M/F)	2.9	3.6	1.3	<0.001
BMI, mean ± SD	25.8 ± 4.1	25.7 ± 4.0	25.7 ± 4.0	0.3
Area of origin				
(i) Northern/Central Italy	423 (44.2%)	349 (44.1%)	74 (45.4%)	0.005
(ii) Southern Italy/Islands	510 (53.3%)	429 (54.2%)	79 (48.5%)	
(iii) Born abroad	24 (2.5%)	14 (1.8%)	10 (6.1%)	
Risk factors				
(i) Alcohol intake alone	459 (48.0%)	374 (47.2%)	85 (52.1%)	0.7
(ii) Alcohol intake/HBsAg positive	63 (6.6%)	54 (6.8%)	9 (5.5%)	
(iii) Alcohol intake/anti-HCV positivity	407 (42.5%)	340 (42.9%)	65 (39.9%)	
(iv) Alcohol intake/HBsAg positive/anti-HCV positive	28 (2.9%)	24 (3.0%)	4 (2.5%)	
Diagnosis				
(i) Liver cirrhosis without HCC	836 (87.4%)	697 (88.0%)	137 (84.0%)	0.2
(ii) Liver cirrhosis with HCC	121 (12.6%)	95 (12.0%)	26 (16.0%)	
Child-Pugh class				
(i) A	381 (45.6%)	307 (44.1%)	74 (54.0%)	0.04
(ii) B/C	455 (54.4%)	390 (55.9%)	63 (46.0%)	

BMI: body mass index. HCC: hepatocellular carcinoma.

of gastric alcohol dehydrogenase and smaller volume of alcohol distribution. In addition, it has been suggested that female hormones increase alcohol mediated liver injury in an unknown fashion [24]. However, males account worldwide for 67% of liver cirrhosis deaths due to alcohol, and world-wide reports (enclosed the present study) have indicated a higher prevalence of alcoholic liver disease in men [2]. This could be because men typically drink more frequently than women do and, more frequently, they are heavy drinkers and alcoholics, regardless of geographic locations [25].

The great decrease of sex ratio from 2001 to 2014 parallels the consistent linear reduction of alcohol intake in the last decade in Italy, particularly among men [6], likely due to both Italian Sanitary Authorities' campaign to prevent alcohol abuse and to economic crisis, which reduces the likelihood of purchase alcohol beverages for money restriction.

The statistically significant younger age of male subjects suggests an earlier in the life exposure to alcohol in this sex. However, the cross-sectional structure of the two studies may have generated a potential survival bias; i.e., men might have died for other diseases more likely than women might do. On the other hand, the biphasic course of liver disease progression in women [26], with a more rapid increase of liver fibrosis in the late menopause than in the repro-ductive/premenopausal/early menopausal status [27], should be also considered. These explanations are not mutually exclusive, but all may have played a role in the observed different age pattern by sex. In any case, the observed ageing over time in both sexes may suggest an improvement of CLD managing over the past 13 years.

The high proportion of alcoholic and anti-HCV positive subjects reflects both the wide spread of HCV infection occurring in Italy after the world war II and the likelihood of behaviours at higher risk of exposure to this infection in these subjects.

A large case-control study showed that two-thirds of decompensated liver cirrhosis in Italy were attributable to alcohol abuse [28]. This figure, even if overestimated for having the authors inaccurately used the proportion of risky alcohol consumers in the hospital controls instead of in the general population for calculating the Population Attributable Risk [29], reflects the tendency of alcoholics to seek medical care only once symptoms are disclosed.

We acknowledge some potential limitations of the present study. Firstly, the history of alcohol intake was retrospectively recorded so that findings may be influenced by a recall bias and by the tendency of alcoholics to minimize or deny alcohol abuse at the time of observation to avoid the negative stigmata associated with alcoholism.

Secondly, some concern for a potential referral bias exists, since about 50% of patients with alcohol abuse were also anti-HCV positive (this condition that may have favoured the enrolment) and the remaining 50% anti-HCV negative often showed a severe liver disease (i.e., liver cirrhosis), a clinical condition that may have favoured the request for medical assistance and the enrolment in the study. This limitation, however, affects any study focusing on alcoholic CLD, as alcoholics tend to refer to hospitals only once severe symptoms have developed.

Thirdly, the definition of risky alcohol intake used in the present study does not capture the pattern of binge drinking, i.e., the consumption of 5 or more drinks in males or 4 or more drinks in women in about two hours [30], a phe-nomenon poorly investigated even in other published studies

although continuously increasing in western countries [31]. Finally, type of beverage has not been assessed; the abuse in drinking wine has been recently shown to be associated with a lower risk of severe liver disease than the abuse in drinking beer and liquor [32].

In conclusion, this survey evidences that risky alcohol intake plays a role in more than one-fifth of CLD in Italy, with a shift over time towards an older age and a more severe disease stage. Considering the decreasing role of chronic HCV [33] and HBV [34] infections in Italy, these data put alcohol back in the spotlight with an important role in CLD in the years to come.

Abbreviations

HCV: Hepatitis C virus
HCC: Hepatocellular carcinoma
anti-HCV: Antibody to HCV
HCV RNA: Hepatitis C virus ribonucleic acid
HBV: Hepatitis B virus
HbsAg: Hepatitis B surface antigen
CLD: Chronic liver disease
PCR: Polymerase chain reaction
ALT: Alanine aminotransferase
US: Ultrasound.

Data Availability

The demographic, epidemiological and clinical data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors of the manuscript declare that they have no conflicts of interest in connection with this paper.

Authors' Contributions

All authors contributed equally to this work, designed the study, and wrote the manuscript.

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Research Article

Ultrasound Grade of Liver Steatosis Is Independently Associated with the Risk of Metabolic Syndrome

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The aim of the study was to explore (a) prevalence and grade of nonalcoholic fatty liver (NAFL) among outpatients referred for abdominal ultrasound (US) examination and (b) relationship between the presence and severity of liver steatosis and metabolic syndrome (MS). This was a retrospective analysis of patients without history of liver disease examined by abdominal US in the University hospital setting. US was used to detect and semiquantitatively grade (0-3) liver steatosis. Data on patients' age, gender, body mass index (BMI), impaired glucose metabolism (IGM), atherogenic dyslipidaemia (AD), raised blood pressure (RBP), transaminases, and platelet counts were obtained from medical records. MS was defined as having at least 3 of the following components: obesity, IGM, AD, and RBP. Of the 631 patients (median age 60 years, median BMI 27.4 kg/m2, and 57.4% females) 71.5% were overweight and 48.5% had NAFL. In the subgroup of 159 patients with available data on the components of MS, patients with higher US grade of steatosis had significantly higher BMI and increased prevalence of obesity, IGM, AD, RBP, and accordingly more frequently had MS, whereas they did not differ in terms of age and gender. NAFL was independently associated with the risk of having MS in a multivariate model adjusted for age, gender, BMI, and IGM. The grade of liver steatosis did not correlate with the presence of liver fibrosis. We demonstrated worrisome prevalence of obesity and NAFL in the outpatient population from our geographic region. NAFL is independently associated with the risk of having MS implying worse prognosis.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined as the fatty infiltration of the hepatocytes exceeding 5% of the liver weight in the absence of other causes such as excessive alcohol intake or hepatitis C [1, 2]. NAFLD encompasses a spectrum of diseases from simple hepatic steatosis (nonalcoholic fatty liver, NAFL) to steatosis with necroinflammatory changes and progressive fibrosis (nonalcoholic steatohepatitis, NASH) [3, 4]. Steatosis is generally a benign condition, whereas NASH can be associated with fibrosis, cirrhosis, and liver failure and carries a higher risk of cardiovascular disease and mortality [2, 4, 5]. NAFLD is often associated with insulin resistance, visceral obesity, excessive body mass index (BMI), type 2 diabetes mellitus (T2DM), hyperlipidemia, arterial hypertension, cardiometabolic alterations, polycystic ovarian syndrome, hypothyroidism, hypogonadism, and sleep apnea abnormalities encompassed by the common term "metabolic syndrome" (MS) [2, 6, 7]. Due to these associations, NAFLD has been until recently considered as the hepatic manifestation of MS [8, 9]. Both MS and NAFLD involve interactions of the adipokines, cytokines, inflammatory factors, and insulin resistance [9]. NAFLD is considered epidemic and serious public health issue with significant impact on the healthcare

expenditures [10]. The prevalence of NAFLD is 2-44% in the general European population (including obese children) and 42.7-69.5% in people with T2DM, with an increase in prevalence with age [11]. Estimates of the worldwide prevalence of NAFLD range from 6.3 to 33% with a median of 20% in the general population [6]. Patients diagnosed with NAFLD have a higher mortality rate when compared to the general population of the same sex and age because of the increased prevalence of cardiovascular disease and increased liver-related mortality rate [12]. A German study showed that subjects with NAFLD as detected by ultrasound (US) and increased serum alanine aminotransferase (ALT) levels had 26% higher overall healthcare costs at 5-year follow-up [13]. Although liver biopsy is the gold standard for diagnosing NAFLD [14], it is not the method of choice to be used in studies that involve general population due to its invasiveness and costs. US, as an alternative tool, is noninvasive, relatively inexpensive, and widely available and increasingly accepted as a method for the initial screening of patients suspected to have NAFLD. According to a meta-analysis, US has pooled sensitivity of 84.8% and specificity of 93.6%, with area under the receiver operating characteristic (ROC) curve of 0.93, to detect moderate-to-severe steatosis (fatty infiltration of >20-30% hepatocytes) [15, 16]. In terms of quantification of liver steatosis, US has recently been challenged by controlled attenuation parameter (CAP), another noninvasive method based on transient elastography (TE) [17, 18]. It has been debated whether the presence and grade of steatosis are to be considered "just" an additional component of the MS or if they have a potential role in the development of MS with prognostic implications [9, 19].

Therefore, the main goals of our study were (a) to assess the prevalence and grade of NAFLD among the cohort of outpatients referred for an US examination and (b) to explore the relationship between the presence and severity of liver steatosis and MS.

2. Patients and Methods

2.1. Study Population. In this study, retrospective analysis was performed over the cohort of consecutive outpatients examined by 3 experienced ultrasonographers in the US Unit of the University Hospital Department of Gastroenterology during a 5-month period.

The study was approved by the Institutional Review Board and was performed in line with the ethical guidelines of the 1975 Helsinki declaration. Upon this approval the following data were retrieved from the patients' medical records: age, sex, BMI, medical history including the history of liver disease, malignancy, diabetes and other forms of impaired glucose metabolism, dyslipidaemia, hypertension, and patients' medications were reviewed looking for the known causes of liver steatosis. All patients gave their verbal consent before US examination. According to national policy only verbal consent is required for US examination provided that no invasive procedures are to be performed.

The exclusion criteria were presence of previously defined hepatobiliary disease other than NAFLD, malignancies, ascites, the use of medications (current or within the last 12 months) known to induce hepatic steatosis (estrogens, corticosteroids, amiodarone, valproate), inflammatory bowel disease, and human immunodeficiency virus (HIV) infection. The following biochemical parameters were documented as well: bilirubin, aspartate aminotransferase (AST), ALT, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and platelet count within 3 months form the US examination. These parameters were used to calculate Fibrosis-4 (FIB4) score as a noninvasive indicator of liver fibrosis stage, since association between severity of liver steatosis and fibrosis has been investigated, and liver fibrosis has been confirmed the most important histological factor in terms of overall mortality and liver-related outcomes [20, 21]. We used dichotomized cut-off values optimized to rule-in (FIB4 ≥2.67; PPV 80%) and rule-out (FIB4≤1.3; NPV 90%) advanced (F≥3) fibrosis [22].

Given the fact that analysis of previous US results demonstrated increasing trend of fatty liver reported in our Unit, we adopted the policy to collect information on patients' body weight and height (parameters needed to calculate BMI) as routine part of the US examination protocol for the purpose of the internal quality control. In addition to this, patients that were found to have fatty liver were routinely asked to quantify their alcohol intake in order to exclude confounding factors in the pathogenesis of hepatic steatosis. We consider excessive alcohol consumption as drinking an intake >40 g/day for men and >20 g/day for women. Therefore, data on patients' age, sex, BMI, alcohol intake, and US findings were available for all patients encompassed by this analysis (N=631, Initial cohort). Since many patients have just undergone abdominal US in our hospital without any further consultancies or blood tests, the number of patients with all parameters available as defined by the study protocol was considerably lower (N=159, Final cohort) than the total number of patients encompassed by initial examination. Study protocol is depicted at Figure 1.

For the purpose of this study we used modified definition of MS as the presence of at least 3 out of 4 of the following components: central obesity, atherogenic dyslipidaemia (AD), raised blood pressure (RBP), and impaired glucose metabolism (IGM). All these have been widely accepted as core components by various definitions of MS in use (World Health Organization (WHO) [23], the European Group for the Study of Insulin Resistance (EGIR) [24], the National Cholesterol Education Program-Third Adult Treatment Panel (NCEP ATP III) [25], and International Diabetes Federation (IDF) [26]). The common components in each of these definitions that are used in this study are defined as follows: (1) central obesity: waist circumference with ethnicity-specific values OR BMI>30 kg/m^2, in which cases central obesity can be assumed and waist circumference does not need to be measured); (2) AD: raised triglycerides (TG): > 150 mg/dL (1.7 mmol/L) OR reduced high-density lipoprotein (HDL) cholesterol: < 40 mg/dL (1.03 mmol/L) in males, < 50 mg/dL (1.29 mmol/L) in females, OR specific treatment for these lipid abnormalities; (3) RBP: systolic BP > 130 or diastolic BP >85 mm Hg, or treatment of previously diagnosed hypertension; (4) IGM: raised fasting

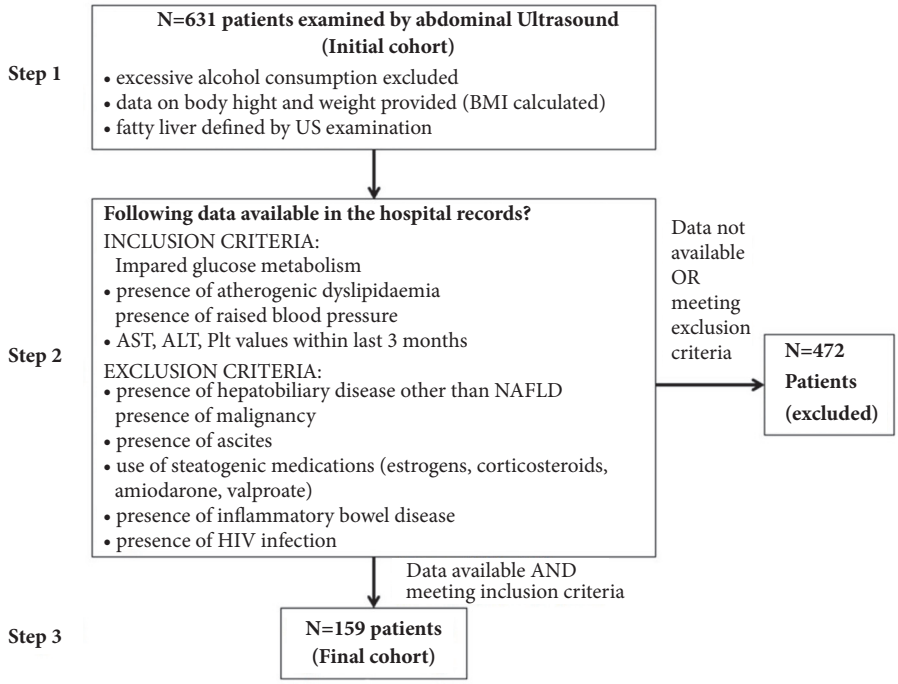

Figure 1: Study protocol. N: number of patients; BMI: body mass index; US: ultrasound; AST: aspartate transaminase; ALT: alanine aminotransferase; Plt: platelet count; NAFLD: nonalcoholic fatty liver disease; HIV: human immunodeficiency virus.

plasma glucose (FPG): >100 mg/dL (5.6 mmol/L), or previously diagnosed T2DM. For the prospective study, sticking to the one of these definitions would certainly be more appropriate. However, since this was retrospective analysis form the available medical records, and some data would have been missing to fit into some of these definitions, we decided to adopt a more a general approach in order to ascertain enough statistical power for the study.

2.2. US Assessment. Abdominal US was performed in patients after overnight fasting, in supine position or in the left decubital position to achieve the best possible visualization of the liver. The US probe was lubricated with gel to avoid artifacts from air and dry skin surfaces. Each US exam was performed by one of three physicians with extensive US experience using an Aixplorer® Ultrasound system, equipped with convex transducer XC6-1 with a 1-6 MHz bandwidth (Supersonic Imagine, Aix-en-Provence, France). Fatty liver on US displayed in the grey scale looks brighter relative to the kidney cortex. With the increased accumulation of liver fat, US waves become more attenuated resulting in a decreased visualization of the deeper parts of the liver (diaphragm and hepatic veins). Diagnosis of fatty liver was based on the increased echogenicity of the liver parenchyma as compared to the right kidney's cortex. Visibility and sharpness of the diaphragm and hepatic veins' interface were analyzed as well. Based on these 3 parameters steatosis was further classified into 3 grades: Grade 0, no steatosis (liver and renal cortex of the same echogenicity); Grade 1, mild steatosis: slightly brighter liver as compared to the renal cortex, clear visualization of diaphragm, and interface of hepatic veins with sharp contours; Grade 2, moderate steatosis: brighter liver with attenuated US beam at deeper parts of the liver, diaphragm, and hepatic veins still visible but with blunted contours; Grade 3, severe steatosis: very bright liver, severe US beam attenuation, diaphragm, or hepatic veins not visible. This classification was adopted and already tested by other investigators [27].

2.3. Statistical Analysis. Normality of distribution of numerical variables was tested using the Kolmogorov-Smirnov test. All numerical variables were nonnormally distributed and were summarized as median and interquartile range (IQR). The Mann–Whitney *U* test/the Kruskal-Wallis analysis of variance (ANOVA) test were used to compare numerical variables between groups where appropriate. Categorical variables were summarized as number and percentage. Jonckhere Terpstra test for trend and the (Chi squared) X^2 test for trend were used to assess trends of increase of tested variables among rising grades of steatosis.

The X^2 test was used to compare categorical variables between groups. The logistic regression was used to investigate associations of categorical variables with other variables while adjusting for potential confounders. P values <0.05 were considered to be statistically significant. Analyses were done using MedCalc Statistical Software version 17.6 (MedCalc Software bvba, Ostend, Belgium).

3. Results

3.1. Demographic Characteristics of Study Participants. A total of 631 patients were encompassed by the primary

Table 1: Demographic and US findings of the entire examined cohort (N=631 patients).

Age (years) (median, IQR)	60 (49-69.75)
Sex (M/F) (N, %)	269 (42.6%) / 362 (57.4%)
Body weight (kg) (median, IQR)	78 (68 - 89)
Height (cm) (median, IQR)	168 (162 - 175)
BMI (kg/m^2) (median, IQR)	27.4 (24.6 - 30.7)
BMI >25 kg/m^2 (N, %)	451 (71.5%)
BMI 25-29.9 kg/m^2	261 (41.4%)
BMI >30 kg/m^2	190 (30.1%)
Liver steatosis (any US grade) (N, %)	306 (48.5%)
US grade 1 steatosis	185 (29.3%)
US grade 2 steatosis	103 (16.3%)
US grade 3 steatosis	18 (2.9%)

IQR: interquartile range; US: ultrasound; BMI: body mass index.

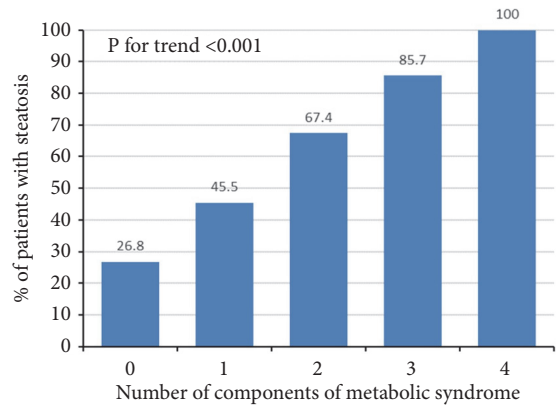

Figure 2: The prevalence of nonalcoholic fatty liver disease (NAFLD) as detected by ultrasound in relationship to the number of components of metabolic syndrome (final cohort, N=159 patients). There is a statistically significant trend of increase in proportion of NAFLD among patients with rising number of metabolic syndrome components, the X^2 test for trend, P<0.001.

analysis. There were 269 (42.6%) males and 362 (57.4%) females; median age was 60 years, IQR (49 – 69.75) years, and median BMI was 27.4, IQR (24.6 – 30.65). Excessive body weight (BMI ≥25 kg/m^2) was recorded in 451/631 (71.5%) patients (41.4% with a BMI 25-29.9 kg/m^2 (overweight) and 30.1% BMI >30 kg/m^2 (obese)). Fatty liver was found in 306/631 (48.5%) patients (US grade 1 in 29.3%, grade 2 in 16.3%, and grade 3 in 2.9% of all patients, with no significant difference between males and females). Demographic characteristics of the patients are presented in Table 1. Patients with fatty liver (N=306) were significantly older (median 61 versus 59 years, p=0.028) and had significantly higher BMI (median 29.7 versus 25.5, p<0.001), but had no difference in terms of gender (45.8% males versus 39.7% females, p=0.124). Prevalence of obesity was significantly higher among patients with, as compared to those without fatty liver (23.1% versus 6.9%, respectively, p<0.001).

3.2. Relationship between Fatty Liver and Metabolic Syndrome. In the second step, we explored the prevalence and the relationship between the components of MS and the presence and US grade of fatty liver.

For the initial cohort of patients (N=631), only the analysis on the relationship between the US grade of liver steatosis and BMI was possible revealing significant increase in BMI over rising grades of liver steatosis (median BMI values of 25.5, 27.8, 31.5, and 36.5 kg/m^2 for grades 0 to 3, respectively, P<0.001). In the post hoc analyses, BMI was significantly different between each grade of steatosis (P<0.05 for all comparisons).

Reliable data on the existence of AD, IGM, and RBP were available for 182 patients, of which 23 met exclusion criteria, and the remaining 159 that formed the final cohort had no conditions known to be capable of inducing fatty liver transformation (see exclusion criteria in Patients and Methods section and Figure 1). Thirty-seven percent of patients in the final cohort were males and 62.3% females, with median age of 59 years, IQR (48.5 – 67.5), and median BMI of 27.3 kg/m^2, IQR (24.4 - 31.5) (69.2% overweight and 34% obese). These results were similar to the initial cohort of 631 patients. AD was present in 65/159 (40.9%), IGM in 34/159 (21.4%), and RBP in 80/159 (50.3%) of patients. In the final cohort, 88 of 159 (53.4%) had fatty liver: 52/88 (59.1%) grade 1, 28/88 (31.8%) grade 2, and 8/88 (9.1%) grade 3. Since only 8 patients had US grade 3 of liver steatosis, for the purpose of further analysis these were merged with patients having US grade 2 steatosis in a single group named "moderate-to-severe steatosis". Fatty liver was detected in 72/110 (65.5%) overweight patients, 26/34 (76.5%) patients with IGM, 47/65 (72.3%) patients with AD, and 56/80 (70%) with RBP. There were no significant differences in age and gender between patients with different US grades of liver steatosis, nor did they significantly differ in liver function tests (Table 2). However, patients with higher US grade of steatosis had significantly higher BMI, increased prevalence of obesity, IGM, AD, and RBP and accordingly significantly more frequently met the modified definition of MS as used in this study (P<0.05 for all analyses) (Table 2). The probability of having NAFLD increased proportionally to the number of components of the MS present in the individual patient (P<0.001) (Figure 2).

US grade of liver steatosis was significantly independently associated with the presence of MS in a series of multivariate models (multiple logistic regression Model 1: nonadjusted model; Model 2: adjusted for age and sex; Model 3: additionally adjusted for BMI; Model 4: additionally adjusted for IGM) as shown in Table 3. Patients with mild liver steatosis had odds ratio (OR) of 5.13 (P=0.07), while patients with moderate-to-severe liver steatosis had OR of 14.68 (P=0.007) for having MS after accounting for aforementioned confounding variables.

Median FIB4 for the final cohort (N=159) was 1.2, IQR (0.8 - 1.6). Only 6/159 (3.8%) patients had FIB4 ≥2.67 indicative of advanced liver fibrosis, 98/159 (61.6%) had FIB4≤1.3, while 55/159 (34.6%) remained between the two values and it was thus not possible to reliably classify them

Table 2: Relationship between ultrasound grade of liver steatosis and clinical features of patients from the final cohort (N=159).

	Normal liver	Mild steatosis	Moderate to severe steatosis	*P*-value for trend
Number	71	52	36	-
Age, years (median, IQR)	60 (47 - 69.5)	56.5 (48 - 67.5)	59.5 (53.25 - 65.25)	0.862
Sex (Male) (N, %)	23 (32.4%)	22 (42.3%)	15 (41.7%)	0.281
BMI, kg/m^2 (median, IQR)	25.4 (22.9 - 27.8)	28.5 (25.2 - 32)	32.6 (28.02 - 35.85)	<0.001∗
Obesity (N, %)	11 (15.5%)	21 (40.4%)	22 (61.1%)	<0.001∗
IGM (N, %)	8 (11.3%)	14 (26.9%)	12 (33.3%)	0.005∗
Atherogenic dyslipidaemia (N, %)	18 (25.4%)	25 (48.1%)	22 (61.1%)	<0.001∗
Raised blood pressure (N, %)	24 (33.8%)	33 (63.5%)	23 (63.9%)	0.001∗
Presence of MS (N, %)	3 (4.2%)	12 (23.1%)	16 (44.4%)	<0.001∗
Number of components of MS (median, IQR)	1 (0 - 1)	2 (1 - 2)	2 (1 - 3)	<0.001∗
AST (IU/L) (median, IQR)	22 (17.5 - 24)	21 (15.8 - 26.5)	24 (20 - 30.25)	0.096
ALT (IU/L) (median, IQR)	19 (13 - 24.5)	20.5 (16 - 26.8)	20.5 (17 - 29.5)	0.051
Bilirubin (mmol/L) (median, IQR)	12 (10 - 14)	13.4 (10.3 - 15.2)	13.1 (10.05 - 15.375)	0.115
GGT (IU/L) (median, IQR)	22 (15 - 35)	24.5 (17.8 - 38.5)	25.5 (18.75 - 33.5)	0.083
ALP (IU/L) (median, IQR)	68 (57.5 - 78)	71.5 (60 - 84)	71.5 (56 - 86.25)	0.334
Plt ($x10^9$/L) (median, IQR)	234 (198.5 - 274)	244 (200.3 - 318)	251 (218.25 - 301)	0.169
FIB4 (IU/L) (median, IQR)	1.2 (0.9 - 1.7)	1 (0.8 - 1.5)	1.2 (1.026 - 1.587)	0.739

∗statistically significant at P<0.05

BMI: body mass index; dyslipidemia: triglycerides>upper limit of normal or high-density lipoprotein (HDL)<lower limit of normal; MS: metabolic syndrome; obesity: BMI>30 kg/m2; IQR: interquartile range; IGM: impaired glucose metabolism; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; Plt: platelet count, FIB4: fibrosis-4 index, RBP: raised blood pressure. Mild steatosis: ultrasound grade 1; moderate to severe steatosis: ultrasound grades 2 and 3.

Table 3: Odds ratios (ORs) for metabolic syndrome regarding degree of NAFLD.

	Mild NAFLD	Moderate to severe NAFLD
Model 1	OR 6.8 95% C.I. [1.81 – 25.56] P=0.005∗	OR 18.13 95% C.I. [4.8 – 68.57] P<0.001∗
Model 2	OR 7.95 95% C.I. [2.03 – 31.13] P=0.003∗	OR 20.58 95% C.I. [5.2 – 81.43] *P*<0.001∗
Model 3	OR 5.31 95% C.I. [1.29 – 21.93] P=0.021∗	OR 9.89 95% C.I. [2.22 – 44] P=0.003∗
Model 4	OR 5.13 95% C.I. [0.89 – 19.71] P=0.070	OR 14.68 95% C.I. [2.08 – 103.67] P=0.007∗

∗statistically significant at P<0.05

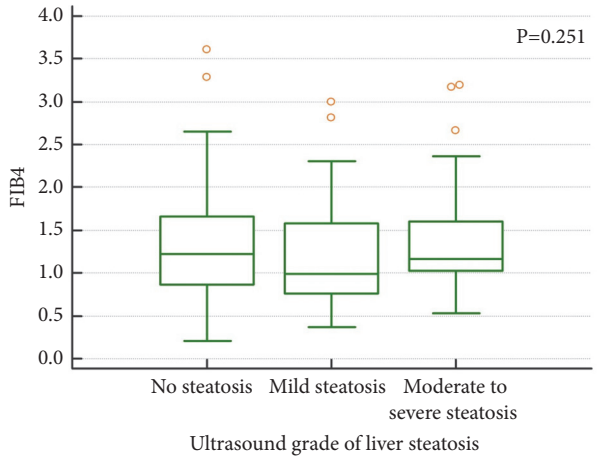

Figure 3: Fibrosis-4 (FIB4) score did not significantly differ between different grades of liver steatosis, the Kruskal-Wallis ANOVA test, P=0.251.

into risk categories for fibrosis. There was no significant difference in individual FIB4 category between patients with and without fatty liver (52/88 (59.1%) patients with fatty liver and 40/71(56.3%) without fatty liver had FIB4≤1.3). Accordingly, there was no significant difference in FIB4 value between different US grades of liver steatosis (p=0.251) (Figure 3).

4. Discussion

In this study, we demonstrated worrisome prevalence of overweight, obesity, and NAFLD in outpatient population from our geographic region. These results are also indicative of the association between higher US grades of liver steatosis and increased risk of having MS and this association was independent from the confounding variables such as age, gender, BMI, and IGM. No significant difference in terms of noninvasively assessed liver fibrosis using FIB4 could be demonstrated between different grades of liver steatosis.

The first important finding of this study is very high prevalence of overweight (41.4%), obesity (30.1%), and NAFL (48.5%) in the analyzed population. These results reveal even worse figures as compared to those reported in the recent European survey showing the prevalence of overweight in 47.6% and obesity in 12.8% of European adults, with respective figures in Croatia being 36.7% and 21.5% [28]. To put these data in the regional context of Central European countries, the corresponding figures of the prevalence of overweight/obesity are 55.6%/9.8% for Hungary, 34.0%/10.5% for Austria, 34.6%/11.7% for Czech Republic, 34.1%/25.4% for Slovakia, and 37.5%/10.3% for Poland [28, 29]. The difference in the reported prevalence of overweight and obesity in Croatian population between the European survey and this study might probably be largely attributed to the selection bias, since our patients are not ideal representatives of the general population. In fact, all of them have been referred to US examination for some medical condition, and it has been well appreciated that overweight and obesity raise the risk for morbidity and overall mortality [30]. Conversely, it is logical to expect higher prevalence of overweight and obesity in people suffering from various health conditions as compared to their lean counterparts. Nevertheless, these figures are worrisome and call for wider action in our community to tackle this growing epidemiological problem. On the other hand, prevalence of MS in our cohort was "only" 19.5%, in contrast to the figures obtained for overweight and obesity. The prevalence of MS is obviously underestimated as anticipated from the study design and especially due to modified definition for MS used here which represents limitation.

The other important finding of this study is that NAFLD patients represent cohort under significant health risk since they have not only high BMI, but also significantly higher prevalence of other individual components of MS. In fact, worsening grades of liver steatosis as detected by US are accompanied by increased prevalence of more individual components and MS itself (Table 3).

Accordingly, patients with more components of MS had higher prevalence of NAFLD (Figure 2). Association

between NAFLD and MS has been recognized before and NAFLD was even considered hepatic manifestation of MS [31]. This concept has been questioned recently as the longitudinal studies provided evidences that NAFLD preceded development of MS [19]. In the elegant population-based cross-sectional study coming from Taiwan, in which another semiquantitative US scoring system was used to grade liver steatosis, the authors demonstrated independent association of liver steatosis and the MS, after adjustment for BMI and insulin resistance as assessed by HOMA-IR [9].

Namely, patients with higher US grades of liver steatosis had increasingly higher OR for MS (3.64 and 9.4 respectively for those with mild and moderate- to-severe NAFLD, as compared to those without NAFLD), which is in line with results obtained in our study. In our cohort association of liver steatosis and MS was consistent over different grades of disease severity in a series of multivariate models adjusted for age, gender, BMI, and IGM. We acknowledge that the result for mild grade steatosis in our final model was of borderline statistical significance. However, clear trend in favor of increased odds of MS with mild disease and clear association of moderate-to-severe disease with MS in our final model are evident. Although limited by retrospective analysis of our data, our study replicates and further supports current concepts of fatty liver as an independent risk factor for MS [9, 19]. In addition, NAFL was found in patients who did not fulfill the criteria of MS and might predict the development of MS or might be a separate pathological entity characterized by a specific genetic predisposition. The latter observation is speculative but might be of use to further investigation of a specific subgroup of patients in future studies.

Concerning the clinical utility of transabdominal US, our data provide evidence that simple semiquantitative scoring of liver steatosis by US reliably predicts severity of metabolic derangements as defined by the increasing number of components of MS. If NAFLD precedes development of MS, its detection would imply the necessity for correction in the early stage by potentially simple intervention such as weight loss. In case when higher grade of steatosis was detected, more comprehensive diagnostic work-up is mandatory to assess the presence and severity of MS. The main limitation of US is the lack of sensitivity to diagnose mild steatosis, as it is capable of diagnosing fatty liver only when at least 20% of hepatocytes have been fatty transformed [16]. Along these lines it should be noted that levels of commonly used biochemical tests such as aminotransferase were not significantly different between patients with different grades of liver steatosis and therefore are obviously not useful for predictive purposes which has already been demonstrated by other authors as well [32, 33].

The final observation from our study is that higher grade of liver steatosis does not impose significant risk for liver fibrosis. This finding should be interpreted with caution due to design of our study and since we used single noninvasive parameter with 1/3 of patients being classified within the grey zone between the values that reliably rule-in (≥2,67) and rule-out (≤1.3) advanced (F≥3) fibrosis. According to current recommendations, two unrelated noninvasive tests (one usually based on US elastography and one biochemical) should be applied to assess the stage of liver fibrosis [34]. In case of disagreement between the two, biopsy is advisable if the result would influence further management of the patient. This concept is important since noninvasive tests are not without limitations. For example, it has been recently demonstrated that higher CAP categories (i.e., more steatotic liver, CAP>300 dB/m) influence the results of liver stiffness measurement (LSM) leading to overestimation of fibrosis especially in the lower range of the fibrosis spectrum [35]. This might have been the reason why significant fibrosis (defined as LSM≥7 kPa) and even cirrhosis (LSM≥10.3 kPa) were observed with a prevalence of 16.7-18.8% and 13.8%, respectively, as reported by the authors who used transient elastography and CAP to assess liver steatosis and fibrosis [36, 37]. It should be also noticed that LSM cut-off values for significant fibrosis and cirrhosis as measured by TE are not the same for different etiologies of liver diseases [38]. By using FIB4, a representative of biochemical tests, the prevalence of advanced fibrosis (F≥3) in our cohort was "only" 3.8%. It is hard to compare these results since we were looking for advanced fibrosis (F≥3), and the other two studies were focused on significant fibrosis and cirrhosis and the inclusion criteria were different.

Furthermore, discrepancies between the reported prevalence of advanced fibrosis/cirrhosis in patients with T2DM (5-7% in UK and 13.8% in Romania) might be at least to some extent attributed to the different methods used to assess liver fibrosis, i.e., biochemical NAFLD fibrosis score (NFS) in the former and TE with CAP in the latter study [39–41]. Our results are in line with the currently prevailing concept that the amount of liver fat is not predictive for the risk of having liver fibrosis [20]. However, this concept has been based mostly on cross-sectional studies, whereas data from the longitudinal studies reveal that gaining the weight and accumulating more liver fat on follow-up biopsy are connected to the higher risk of fibrosis progression [20]. On the other hand, in the Rotterdam study that included 3,041 participants from the general population, steatosis as detected by US was strongly associated with the presence of clinically relevant fibrosis (defined as LSM≥8 kPa by TE [42], with the prevalence of 5.6%). Since no histology data were provided, these results might suffer from the same limitation as previously mentioned studies due to the possible overestimation of fibrosis in patients with more severe steatosis.

Apart from liver-related risks, higher grades of liver steatosis are related to the increased risk of cardiovascular morbidity [43, 44].

The main limitation of our study is its retrospective approach. For this reason certain data that were needed to meet one of the established definitions of MS were not available from the medical records precluding formation of the uniform cohort with significant power to perform statistical analysis. Thus, for the purpose of this study, MS was considered in patients who had at least 3 (any 3) out of 4 core components contained in any established definition of MS. Since dyslipidaemia was defined by either elevated

TG or low HDL values or specific medication used for these conditions (similar to WHO and EGIR definition), it might have underestimated the real prevalence of MS, as cases with both lipid components were not recognized by this definition. Along this line, overlapping between high TG and low HDL might have been presumed especially in obese patients already commenced to lipid-lowering therapy, even though this overlapping was not evident from the biochemical data available at the study inception. Further limitation refers to the liver biopsy which was not available, and our results rely on previous studies that demonstrated good correlation between the US and histological diagnosis and grading of liver steatosis. The same limitation stands for the assessment of liver fibrosis, which was performed by calculating only FIB4 score in our study.

In conclusion, results of this study demonstrate high prevalence of overweight, obesity, and NAFLD in the outpatient population in our geographic region.

Patients with higher US grades of liver steatosis are under increased risk of MS independently of age, gender, BMI, and IGM, but not of liver fibrosis. Simple semiquantitative US scoring of liver steatosis might help in earlier recognition of MS and enable timely interventions aimed to reduce cardiovascular risk, therefore improving prognosis of these patients.

Data Availability

The raw data obtained by the authors which were used for all calculations and the results of this study were submitted as a supplementary file with the manuscript.

Ethical Approval

This study was approved by the Institutional Review Board and was performed in line with the ethical guidelines of the 1975 Helsinki Declaration.

Disclosure

No specific funding was received for the study. Research was performed as part of the employment of the authors at University Hospital Dubrava, Zagreb.

Conflicts of Interest

All authors declare no conflicts of interest related to this article.

Authors' Contributions

Sanda Mustapic contributed to acquisition and analysis of data and drafting the manuscript. Sead Ziga, Vladimir Matic, and Tomislav Bokun assisted in acquisition and analysis of data and revising the manuscript critically. Marko Lucijanic helped in analysis and interpretation of data and drafting the manuscript. Bozo Radic, Zarko Babic, and Srecko Marusic contributed to analysis and interpretation of data and revising the manuscript critically. Ivica Grgurevic contributed to conception and design of the study, acquisition, analysis, and interpretation of data, and drafting the manuscript,. All the authors approved the final version of the article, including the authorship list

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Review Article

Chronic Hepatitis C Association with Diabetes Mellitus and Cardiovascular Risk in the Era of DAA Therapy

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Patients with chronic hepatitis C have both higher prevalence of diabetes mellitus type 2 (T2DM) and increased cardiovascular risk compared to never infected people. Sustained viral response (SVR) achievement led to decreasing incidence and prevalence of T2DM during the interferon era of HCV treatment. Currently, direct-acting antiviral drugs (DAA) are the gold standard for treating HCV infection, while yielding SVR in nearly all patients. In chronic HCV patients with T2DM (prediabetes most likely too), DAA therapy is associated with both better fasting glucose and glycated hemoglobin (HbA1C) controls; thus reducing pharmacotherapy in a certain part of patients is possible. Papers mentioned in the review confirmed DAA role in both total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) increase. This alteration was accompanied by an increase in high-density lipoprotein cholesterol (HDL-C) and a decrease in triglycerides (TG) verified by most of the studies. However, the clinical significance of lipoprotein alterations caused by DAA therapy has not been explained yet. Moreover, DAA treatment of chronic hepatitis C improves hypertension control and atherosclerotic plaques. It is very likely that DAA therapeutic regimens will decrease both T2DM prevalence and cardiovascular risk in chronic hepatitis C patients; further research, however, is needed.

1. Introduction

Chronic hepatitis C virus (HCV) infection affected some 170 million people worldwide in 2013 [1]. According to the latest information, in the view of better screening, diagnostics, and discovery of effective therapeutic regimens, the hepatitis C virus prevalence has been decreasing to currently 70 million people worldwide [2]. Chronic HCV infection tends to progress to liver fibrosis and cirrhosis. Subsequently, hepatocellular carcinoma can develop in the context of bridging fibrosis (F3 by Metavir) or liver cirrhosis (F4 by Metavir).

Decompensated liver cirrhosis together with hepatocellular carcinoma is the most common cause of death associated with chronic HCV infection [3].

Nowadays, chronic HCV infection is considered a systemic disease, while it does not affect only the liver, but other organs as well. Nearly three-quarters of patients also suffer from extrahepatic manifestations, which can already be seen before the diagnosis of chronic HCV infection [4]. Diabetes mellitus type 2 (T2DM) is one of the most common extrahepatic manifestations of chronic HCV infection [5]. Moreover, HCV accelerates atherogenesis and is also associated with cardiovascular disorders [6]. HCV infection increases not only liver disease mortality rate but also cardiovascular and all-cause mortality rate [7].

The primary goal of chronic HCV infection treatment is to achieve sustained viral response (SVR), characterized by the complete disappearance of hepatitis C virus from patient's body. SVR is associated with decreased liver disease mortality rate together with all-cause mortality rate [8]. Recently, there has been a remarkable breakthrough in the treatment of chronic HCV infection, in the form of the implementation of direct-acting antivirals (DAA) into clinical practice guidelines. Using a combination of at least two of DAA, specifically NS5A inhibitor, NS5B inhibitor, or NS3/4a protease inhibitor, results in a very high response rate [9].

This review aims to briefly describe the association between insulin resistance, T2DM, atherogenesis, and cardiocerebrovascular disorders on one hand and chronic HCV infection on the other. We also present the impact of the DAA therapy on glycide and lipoprotein metabolism, together with possible implications of the DAA therapy on cardio- and cerebrovascular risk.

2. Chronic HCV Infection, Insulin Resistance, and T2DM

Chronic HCV infection can lead to increased insulin resistance as hepatitis C virus impairs the hepatocyte insulin signaling pathway in multiple ways [10], including (i) increased production of tumor necrosis factor-α, (ii) phosphorylation of the insulin receptors, (iii) the overexpression of the suppressor of cytokines (SOC-3), and (iv) induction of SOC-7 [11–13]. Even in HCV infected patients without metabolic syndrome, not only liver, but also whole-body insulin sensitivity is impaired. It is assumed that HCV infected liver produces mediators, which cause increased insulin resistance at extrahepatic sites, mainly in skeletal muscles.

Increased insulin resistance plays pivotal role in the development of T2DM in patients with chronic HCV infection [5, 14, 15]. In fact, insulin resistance is already present in patients with chronic HCV infection with low-grade fibrosis and its prevalence is significantly higher in infected than in healthy controls population. Also, its presence positively correlates with the grade of fibrosis and portal inflammation [16]. Furthermore, insulin resistance increases the prevalence of hepatocellular carcinoma and cardiovascular events in patients with chronic HCV infection [17].

Pathological background of T2DM is insulin resistance. T2DM is one of the civilization diseases and, recently, its prevalence has been showing an increasing tendency. Currently, 350 million people are suffering from this disease worldwide [1]. In industrialized countries, its prevalence is even higher with overall estimated prevalence some 9% in Europe [18].

T2DM is a disease with severe socioeconomic consequences and leads to decreased life expectancy, particularly when diagnosed early in life [19].

Prediabetes is approximately four times more frequent in patients with chronic HCV infection than in healthy controls population. Predisposing factors for prediabetes are both older age and higher ALT levels [20]. The assumption is that one-third of the patients with chronic HCV infection could have T2DM [21]. According to meta-analysis, patients infected with HCV are at higher risk for development of T2DM than noninfected patients (OR: 1.68; 95% CI: 1.15-2.45) [22]. Another meta-analysis showed similar results both in retrospective (adjusted OR: 1.68; 95% CI: 1.15-2.20) and prospective (adjusted HR: 1.67; 95% CI: 1.28–2.06) studies. Moreover, a group of patients with HCV/HIV coinfection has increased T2DM prevalence than a group of patients infected with HIV exclusively (OR: 1.82; 95% CI: 1.27–2.38) [23]. Among patients with hepatitis C, male gender (OR: 1.26, 95% CI: 1.03-1.54) and age over 40 years (OR: 7.39, 95% CI: 3.82-9.38) had higher prevalence of T2DM [22]. Furthermore, according to other study, higher BMI, F4 at the elastographic examination of the liver, duration of hepatitis C infection, response to previous therapy, and positive family history for T2DM together with insulin sensitivity can predict the development of T2DM [1]. Prevalence of chronic HCV infection is higher in patients with T2DM compared to nondiabetic patients [24]. The prevalence of T2DM in a group of chronic HCV infected patients with liver cirrhosis is higher than in both patients not suffering from liver disease (adjusted RR: 8.71; 95% CI: 1.28-59.46) and patients with liver cirrhosis of other causes (adjusted RR: 2.03; 95% CI: 1.54-2.67). Predictive factor for T2DM development in HCV positive patients with liver cirrhosis is albumin level less than 39 grams per liter [25]. In HCV patients, new onset diabetes predicts decompensation of liver cirrhosis (RR: 2.01; 95% CI: 1.07-3.79; $p<0.001$) [26]. Interestingly, the incidence of diabetic retinopathy in patients with liver cirrhosis is significantly lower in HCV positive than in HCV negative patients [27, 28]. T2DM is considered to be accelerating carcinogenesis in patients with chronic HCV infection. There is a higher prevalence of hepatocellular carcinoma in the group of HCV positive patients with T2DM than in the group of HCV positive patients without T2DM [29].

Eventually, achievement of SVR could lead to a drop in the prevalence of T2DM in patients with chronic HCV infection in the near future. Insulin resistance leads to a worse therapeutic response to insulin therapy in patients with chronic HCV infection [30]. There are four studies which evaluated the effect of SVR achievement on T2DM incidence during the era of interferon therapy. A retrospective study from Japan followed 2 842 patients with chronic HCV infection treated with interferon therapy. The average duration of the follow-up was 6.4 years. The cumulative prevalence of T2DM was 3.6% at five years, 8.0% at ten years, and 17.0% at 15 years. Predictive factors for T2DM development were advanced liver disease (HR:3.30; 95%CI: 2.06-5.28; $p < 0.001$), failure to achieve SVR after therapy (HR: 2.73; 95%CI:1.77-4.20; $p < 0.001$), baseline prediabetes (HR: 2.19; 95%CI: 1.43-3.37; $p < 0.001$), and age ≥ 50 years (HR: 2.10; 95%CI: 1.38-3.18; $p < 0.001$) [31].

A prospective study from Spain followed 1 059 patients with chronic HCV infection treated with interferon therapy. Insulin resistance was a negative predictive factor for SVR achievement. SVR achievement (OR: 0.44; 95%CI: 0.20-0.97; $p=0.004$) together with fibrosis stage (OR: 1.46; 95%CI: 1.06-2.01; $p=0.02$) was defined as independent risk factors for both development of impaired fasting glucose and T2DM by logistic regression analysis [32].

Another retrospective study from Spain followed 234 patients. All patients had chronic HCV infection, neither had liver cirrhosis, and all of them were treated with interferon therapy.

Those patients, who had been able to achieve SVR, were in lower risk for development of glucose abnormalities (HR:

0.48; 95%CI: 0.24-0.98, p = 0.04) [33]. Lastly, in a retrospective study from Italy, there was no association described between the achievement of SVR and lower risk of developing T2DM. Patients in this study had been followed for over eight years. However, one should take into consideration the limitation represented by a very low number of patients followed in this study [34].

New diabetes mellitus treatment options discovered recently could improve glycemic and metabolic profile and cardiovascular risk [35]. Furthermore, recent prospective study showed that SGLT2 inhibitor for NAFLD complicated by T2DM improved hepatocyte steatosis and liver fibrosis [36]. New antidiabetic drugs introduced into the clinical practice currently and in the future will improve glycemic control in T2DM patients with chronic hepatitis C.

3. Chronic Hepatitis C and Lipoprotein Metabolism

HCV assembly and secretion are closely associated with synthesis and secretion of lipoproteins. Entry of HCV particles into hepatocyte is dependent on lipoproteins with apolipoproteins playing the principal role. Thus, lipoprotein-HCV interaction directly affects infectivity of HCV particles [37]. HCVs are secreted from hepatocyte as highly infective lipoviral particles, which contain mainly apolipoprotein C and apolipoprotein E [38].

Therefore, lipoproteins play the crucial role in HCV infectivity.

Chronic HCV infection is associated with the presence of fatty liver disease [6]. Fatty liver is approximately five times more frequent in genotype 3a than in genotype 1. It is not associated with BMI values nor ferritin values; on the other hand, it is very closely associated with HCV viral load. Patients with HCV genotype 3a have significantly lower total cholesterol values (TC) than patients with genotype 1 [39]. This type of lipid accumulation in the liver is called viral steatosis. There is no increased insulin resistance with viral steatosis, it does not lead to progression of liver fibrosis, and it does not affect the impact of interferon therapy. Viral steatosis vanishes after achieving therapeutic response and appears back during relapse of chronic hepatitis [6, 39, 40]. The association between viral steatosis and atherogenesis acceleration has not been studied yet. On the other hand, metabolic steatosis in chronic HCV infection is associated with insulin resistance, accelerates atherogenesis, leads to liver fibrosis progression, and worsens interferon therapeutic response. Metabolic steatosis and viral steatosis most likely also increase the risk of hepatocellular carcinoma in patients with chronic HCV infection [6].

Typical laboratory abnormality in HCV infected patients is viral hypolipidemia. There are lower TC and LDL-C levels in patients with chronic HCV infection than in noninfected patients. However, HDL-C and triacylglycerides levels are roughly the same in both groups of patients. Though, after achieving viral response, viral hypolipidemia disappears [41].

Hyperlipidemia in patients with chronic HCV infections is rather rare. Out of 280 patients with chronic HCV infection and thalassemia, only one had higher LDL-C levels (0.4%), and 19 had higher levels of TG (7%) [42]. Egyptian study describes lower TC, LDL-C, and TG levels in patients with chronic HCV infection than in noninfected patients. In contrast, participants who cleared HCV infection had higher triglyceride levels compared with those never infected. The question that remains to be answered is whether higher TG level in patients with chronic HCV infection does or does not increase the chance for spontaneous HCV clearance [43, 44].

Infected patients do not have atherogenic dyslipidemia. This fact is the reason why the incidence of metabolic syndrome is, after sex, gender, and fibrosis stage adjustment, not higher in infected patients than in healthy controls [45]. In spite of that, patients with chronic HCV infection are at higher cardiovascular risk than noninfected patients [46, 47]. The reason of that could be explained by studying individual fractions of lipoproteins. Japanese authors found that patients with chronic HCV infection, genotype 1, and advanced fibrosis had higher levels of LDL-TG, HDL-TG, and small VLDL-TG. LDL-TG and small VLDL-TG are responsible for the acceleration of atherogenesis. It becomes one of the possible explanations for atherogenesis acceleration in patients with chronic HCV infection and advanced liver fibrosis, albeit further research on the association between altered lipoprotein metabolism and accelerated atherogenesis in chronic HCV patients is needed [48].

Although hyperlipidemia with chronic hepatitis C is a rare finding, patients could benefit from statin therapy, while they have several pleiotropic effects in hepatology [49]. The addition of statins to pegylated interferon and ribavirin therapy increases the chance of achieving SVR (OR: 2,02; 95%CI 1,38-1,94) [50]. Statins retard hepatic fibrogenesis, mainly through reducing of microthrombus formation in hepatic circulation [49]. In the HALT-C study, statins reduced risk of fibrosis progression in nonresponders to pegylated interferon and ribavirin therapy (HR: 0,32; 95% CI 0,10-0,99) [51]. Statin use was also associated with a reduced risk of liver cirrhosis development in a dose-dependent manner among patients with chronic HCV infection compared to patients not treated with statins [52]. There is a possibility that statins could reduce the risk of hepatocellular carcinoma development in patients with HCV hepatitis. ERCHIVES study described the ability of statins to reduce the incidence of hepatocellular carcinoma among patients with chronic HCV infection (aHR: 0,60, 95%CI 0,53-0,68). This effect was time-dependent, and Rosuvastatin and Fluvastatin showed the best efficacy [53]. After all, there is no knowledge about how statins do influence atherogenesis and cardiovascular risk in HCV patients, yet.

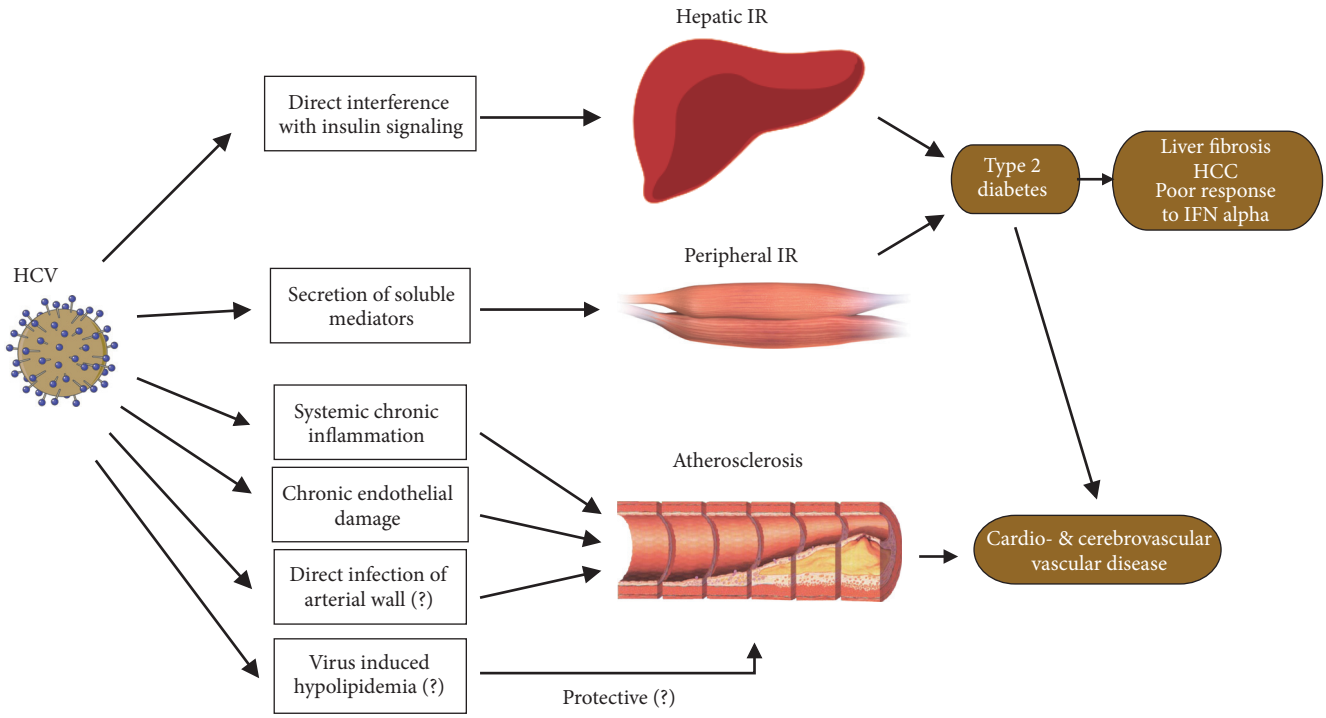

Figure 1: Schematic representation of interactions between hepatitis C virus and cardiovascular risk. Adapted from Negro 2014 [6].

4. Chronic Hepatitis C and Cardiovascular Risk

Although the association of chronic HCV infection with higher cardiovascular risk has not been confirmed yet, the majority of the research papers consider higher cardiovascular risk as one of the extrahepatic manifestations of HCV infection. The higher cardiovascular risk in HCV infection has multifactorial pathogenesis. As shown above, HCV infected patients have higher prevalence and risk of development of T2DM and its association with accelerated atherogenesis is well known. Furthermore, chronic HCV infection also accelerates atherogenesis by direct pathological pathways: (i) chronic systemic inflammation, (ii) chronic endothelial damage, and (iii) direct infection of the arterial wall.

T2DM together with accelerated atherogenesis increases cardio-cerebrovascular risk [6]. The question that we are still looking for the answer to is whether hypolipoproteinemia mentioned in the previous chapter could have a protective effect on atherogenesis (see Figure 1).

Atherosclerosis leads to both formation of atherosclerotic plaques in arteries and increased arterial intima-media thickness. Both carotid artery plaques and carotid intima-media thickness (cIMT) are subjects of studies. Chinese researchers published meta-analysis of eight studies. In seven of them, HCV infection significantly increased the risk of carotid atherosclerosis compared to those never infected (adjusted OR: 1.76, 95%CI: 1.20-2.32) [76]. Two other studies shed light on the association of HCV seropositivity with coronary atherosclerosis. In the Turkish paper, HCV seropositivity was an independent predictor for severity of coronary atherosclerosis (OR: 2.02; (95%CI: 1.58-2.58, $p<0.001$) [77]. On the other hand, in the research performed in Japan, prevalence of anti-HCV antibodies in patients without coronary artery disease was 2.8% and in patients with coronary artery disease only slightly higher, 3.4%, although limitation to this research was a low number of examined patients [78].

Angina pectoris, myocardial infarction, and stroke are some of the most frequent signs of atherosclerosis. In a meta-analysis of 34 studies, all of which followed patients with coronary artery disease, unstable angina pectoris, myocardial infarction, and stroke, patients with chronic HCV infection were in significantly higher risk for cardio-cerebrovascular disease than noninfected patients (OR: 1.43; 95%CI: 1.21 - 1.68). Meta-analysis of 22 studies found the substantially higher risk for coronary artery disease in patients with chronic hepatitis C than controls (OR: 1.38; 95%CI: 1.10-1.73) [46]. Studies we mentioned previously had proved that chronic HCV infection raises the risk of both subclinical and clinically apparent cardio-cerebrovascular disease.

5. DAA Therapy Effect on Glycemia and Glycated Hemoglobin

Studies performed during the era of interferon therapy of chronic hepatitis C revealed a significant drop in fasting

glucose and glycated hemoglobin (HbA1C) levels when patients achieved SVR. This drop was not observed in patients with chronic hepatitis C relapse [79]. Majority of papers evaluating the effect of DAA on fasting glucose or HbA1C followed patients with both chronic HCV infection and diabetes mellitus. Post hoc analysis of 6 studies that followed patients in 3a stage of chronic hepatitis C, genotype 1, treated with 3D combo paritaprevir/ritonavir + dasabuvir + ombitasvir, revealed that 68.7% of all patients included in the study had normal glycemia levels, 25.4% of patients had prediabetes, and 5.9% of patients had diabetes mellitus. There was a significant drop in fasting glucose in the group of patients who received treatment compared to the group that received placebo. In overall, notable drop in fasting glucose was observed (–8.87 mg/dL by week 12; $p < 0.0001$). The most significant drop in fasting glucose was recorded in the group of patients with T2DM (–22.1 mg/dL by week 12; $p < 0.0001$), followed by still significant drop of fasting glucose in the group of patients with prediabetes (–5.78 mg/dL by week 12; $p < 0.0001$). On the contrary, there was slight, not significant increase of fasting glucose in the group of patients with normal baseline fasting glucose levels (1.34 mg/dL by week 12; $p = 0.057$) [54].

Further studies assessed decrease in fasting glucose or HbA1C during therapy or after the completion of DAA therapy in patients with chronic hepatitis C and T2DM. Two of them followed patients with genotype 4 exclusively [63, 64] and the third followed a group of patients, where the majority of patients were infected with genotype 4 [62]. All three above-mentioned studies dealt with Egyptian population. A Japanese study followed chronic hepatitis C patients with genotype 1b exclusively [55]; another study observed patients with genotype 1, exclusively [56]. The remaining studies followed patients regardless genotype, while genotype 1 dominated [57–61]. Different treatment options were studied. Morales et al. used the combination of pegylated interferon, sofosbuvir, and ribavirin [58]. All other studies employed interferon-free regimens. Meissner et al. used sofosbuvir and ribavirin [56], Egyptian study used sofosbuvir and daclatasvir [63], another Egyptian study used sofosbuvir and simeprevir [64], and Japanese study used sofosbuvir and ledipasvir [55]. In two studies, the patients were not treated uniformly, though always sofosbuvir was used [58, 62]. In three Italian studies patients were treated mostly with combinations based on sofosbuvir, only small part of them was treated with paritaprevir/ritonavir + dasabuvir + ombitasvir [59–61].

Research performed in the US followed 2 435 patients from National Veterans Affairs healthcare system. They were treated with sofosbuvir and simeprevir, sofosbuvir and ledipasvir, or the combination of paritaprevir/ritonavir + dasabuvir + ombitasvir. None of them received ribavirin [57].

Both fasting glucose and HbA1C dynamics were evaluated in four analyses [59, 61–63], two studies evaluated changes in fasting glucose solely [60, 64], and four studies reported HbA1C dynamics only [55–58]. All of them observed significant drop in fasting glucose levels or HbA1C during or after the completion of therapy [55–64].

New Zealand study followed patients after liver transplantation. Out of 91 treated patients, 62 were nonresponders on previous therapy. More than half of them were infected with hepatitis C virus genotype 1. Majority of patients received combination based on sofosbuvir; three patients received a combination of paritaprevir/ritonavir + dasabuvir + ombitasvir, and six patients were treated with glecaprevir + pibrentasvir. Out of all patients, 96% achieved SVR. HbA1C values dropped from 35.5±4.3mmol/mol to 33.3±3.6 mmol/mol at 44 weeks after treatment ($p = 0.03$). Those patients, who were not treated with antidiabetics, were observed with fasting glucose level drop from 6.8±1.7mmol/L before therapy to 5.7±1.1mmol/L 24 weeks after completion of therapy [65].

In contrast to that, a prospective study followed 251 patients with chronic HCV infection, genotype 1 a/b. Out of all patients, 31% were HIV positive, and 17% of patients had T2DM, out of whom 79% were treated with antidiabetic therapy. One patient was treated with pegylated interferon, ribavirin, and telaprevir; other patients were treated with various antivirotics, including sofosbuvir, ledipasvir, beclabuvir, daclatasvir, and asunaprevir. Contrary to the previous study, after completion of therapy, HbA1C levels did not differ in patients who achieved SVR from those patients who failed to achieve SVR. HbA1C drop in patients with SVR was 0.022±0.53% (NS). Also, changes in HbA1C levels after completion of therapy were roughly the same in the group of HCV/HIV coinfected patients with SVR and in the group of HCV/HIV coinfected patients without SVR. Moreover, HbA1C levels did not differ between a group of diabetic patients with SVR and group of diabetic patients without SVR after completion of therapy. The limitation of this study was a low number of patients with T2DM comorbidity, and different DAA regimens used [66]. The results of the studies mentioned above are summarized in Table 1.

The drop in fasting glucose was not observed in all patients. Italian study found a drop in fasting glucose levels in 67% patients and a drop in HbA1C in 80% of patients with chronic hepatitis C and T2DM [61]. Egyptian paper analyzed patients with chronic hepatitis C, genotype 4, and T2DM. Every patient achieved SVR. Drop in glycemia levels was observed in 77.2% patients 12 weeks after therapy. Prognostic factors for a drop of glycemia levels >20 mg/dl or a drop of HbA1C levels > 0,5% were identified in multiple logistic regression analysis. Prognostic factors were the duration of T2DM < 7 years, negative family history for T2DM, or any Child-Pugh A stage of liver disease [63]. Analysis based on National Veterans Affairs healthcare system database followed patients with both chronic hepatitis C and T2DM. The conclusion of this analysis describes a significant drop in HbA1C in patients who achieved SVR compared to patients with failure to achieve SVR [57].

One of the severe complications of T2DM during DAA therapy is hypoglycemia. Spanish authors published case report of a well-compensated diabetic patient before sofosbuvir + ledipasvir therapy. The patient received 18 units of basal insulin daily and every six to eight hours 4-8

Table 1: Studies reporting the changes of fasting glucose, HbA1C, and antidiabetic treatment after DAA treatment.

Author	Country	Patients	Genotype	Treatment	Decrease of the fasting glucose during or after DAA treatment	Decrease of the HbA1C during or after DAA treatment	Proportion of patients with the reduction of antidiabetic treatment
Tran,2017 [54]	Multi-ethnic	General HCV population, 25.4% prediabetes 5.9% T2DM	Genotype 1	Paritaprevir/ ritonavir + dasabuvir + ombitasvir	Yes, in all patients, patients with prediabetes and T2DM	NA	NA
Ikeda, 2017 [55]	Japan	T2DM	Genotype 1b	Sofosbuvir + ledipasvir	NA	Yes	NA
Meissner, 2015 [56]	USA	T2DM	Genotype 1	Sofosbuvir + ribavirin	NA	Yes	NA
Hum [57]	USA	T2DM	Mostly genotype 1	Sofosbuvir + simeprevir or Sofosbuvir + ledipasvir or Paritaprevit/ritonavir + dasabuvir + ombitasvir	NA	Yes	9%
Morales, 2016 [58]	USA	T2DM	Mostly genotype 1	Only sofosbuvir based	NA	Yes	25%
Ciancio, 2018 [59]	Italy	T2DM	Mostly genotype 1	Mostly sofosbuvir based	Yes	Yes	21%
Fabrizio, 2017 [60]	Italy	T2DM	Mostly genotype 1	Mostly sofosbuvir based	Yes	NA	NA
Pavone, 2016 [61]	Italy	T2DM	Mostly genotype 1	Mostly sofosbuvir based	Yes	Yes	23%
Abdel Alem, 2017 [62]	Egypt	T2DM	Mostly genotype 4	Only sofosbuvir based	Yes	Yes	NA
Dawood, 2017 [63]	Egypt	T2DM	Genotype 4	Sofosbuvir + daclatasvir	Yes	Yes	27%
El Sagher, 2018 [64]	Egypt	T2DM	Genotype 4	Sofosbuvir + simeprevir	Yes	NA	NA
Beig, 2018 [65]	New Zealand	LTx patients, only patients without antidiabetic treatment	Mostly genotype 1	Mostly sofosbuvir based	yes	Yes	40%
Chaudhury, 2017 [66]	USA	General population, 31% HIV positive, 17%T2DM	Genotype 1	Multiple DAA	NA	No	3%

NA: not available; T2DM: type 2 diabetes mellitus.

units of bolus insulin based on glycemia. HbA1C was 6.5%. From the 7th day of therapy on, his bolus insulin dose was reduced. Despite that, on the 18th day of the therapy, the patient presented with symptomatic hypoglycemia with glucose level 50 mg/dL. On the 21st day of the therapy, bolus insulin was discontinued, and later on, also basal insulin was discontinued. The decreased demand for insulin came with ALT normalization and HCV RNA disappearance from the patient's serum [80]. Thus, 3-40% of patients treated with antidiabetics are in a need of dose reduction during DAA therapy, while patients treated with insulin need dose reduction even more frequently [55, 57, 59, 61, 63, 65, 66].

All of the previously mentioned findings confirmed better compensation of diabetes mellitus in patients who are treated with DAA. Majority of studies included patients treated with therapeutic regimens based on sofosbuvir.

Reducing antidiabetic therapy in a certain part of patients is possible.

6. The Effect of DAA on the Lipoprotein Metabolism

HCV life cycle requires lipoprotein particles. Therefore, alteration of lipoprotein profile after SVR achievement is possible. Austrian scientists described a significant increase in TC after SVR achievement in patients with genotype 3a. In contrast to that, patients with genotype 3a with failure to achieve SVR did not present with increase in TC values [39]. There are several papers concerning the effect of DAA on the lipoprotein metabolism. Three studies followed patients with genotype 1b exclusively [68–70], five studies dealt with patients with genotype 1 exclusively [56, 66, 67, 71, 72], and another five studies followed mostly genotype 1 [58, 65, 73–75].

Egyptian study followed patients particularly with genotype 4 [64]. One study dealt with patients after liver transplantation exclusively and another was concerned with coinfected patients [72]. Three studies dealt also with patients treated with interferon [58, 73, 75]. One study used combination of sofosbuvir + ribavirin [56], and another used combination of sofosbuvir + simeprevir [64]. Combination of daclatasvir + asunaprevir was applied also [68]. Two studies treated patients either with sofosbuvir + ledipasir or daclatasvir + asunaprevir [69, 71], and one research applied combinations of either sofosbuvir + ledipasir, daclatasvir + asunaprevir, or sofosbuvir + ribavirin [70]. Furthermore, combinations of sofosbuvir + ledipasir or grazoprevir + elbasvir were applied [67], one study used only sofosbuvir based therapy [58], and four studies treated patients mostly with sofosbuvir based combinations [65, 72–74]. Moreover, two studies used different combinations of DAA [66, 75].

Conclusions of above-mentioned studies were as follows. One study observed an increase in TC (LDL-C was not assessed) [67], and another observed an increase in LDL-C (TC was not assessed) [56]. In the 12 remaining studies increase of both TC and LDL-C was recorded [58, 64–66, 68–75]. Three studies described a significantly higher increase in both TC and LDL-C when the combination of sofosbuvir + ledipasvir was used compared to the combination of daclatasvir + asunaprevir [68–70]. Some of the studies mentioned above assessed HDL-C dynamics also. Four papers described an increase in HDL-C during or after treatment [64, 68, 69, 72], and two papers observed no alteration in HDL-C [65, 73]. Furthermore, seven studies assessed TG during and after treatment. Four papers described a decrease in TG [56, 66, 67, 70], although three studies observed no changes in TG [65, 72, 73]. One study dealt with TG dynamics during the therapy with the combination of paritaprevir/ritonavir + dasabuvir + ombitasvir. There was a significant drop in TG compared to the group of patients who received placebo. The most significant drop was observed in the group of patients who had presented with hypertriglyceridemia before the therapy. In contrast to that, there was a small, but still significant, increase in hypertriglyceridemia in the group of patients presenting with normal triglycerides levels before the therapy [54]. The studies on the lipoprotein metabolism are summarized in Table 2. Japanese authors described both significant increase in lipoprotein(a) and alteration of apolipoprotein B/apolipoprotein A1 ratio, in chronic hepatitis C patients with genotype 1 after the completion of DAA treatment [74]. A study from the US observed a significant drop in both apolipoprotein AII and apolipoprotein E and a significant increase in apolipoprotein C in chronic hepatitis CII patients with genotype 1 after DAA treatment [81].

Considering all of the studies mentioned above, the effect of DAA therapy on atherogenesis after achieving SVR is hard to assess. There is a significant increase in both TC and LDL-C on one side and a considerable increase of HDL-C together with a considerable decrease of TG, on the other. Further research, with a high number of patients along with lipoprotein fractions and subfractions dynamics assessment, is needed regarding the effect of lipoprotein metabolism alterations on atherogenesis.

Importantly, besides the effect on glycemia and lipoprotein metabolism, DAA treatment affects atherogenesis by other means as well. According to the latest information, SVR achievement after DAA therapy improves carotid atherosclerosis directly [82]. Moreover, New Zealand study observed blood pressure improvement in patients after liver transplantation [65].

7. Conclusion

Chronic hepatitis C is associated with both the development of insulin resistance and T2DM. In spite of viral hypolipidemia, infected patients are at higher cardiovascular risk. The positive effect of SVR achievement on decreasing incidence and prevalence of T2DM was proved already during the interferon era of HCV treatment. DAA therapy of chronic HCV infection is yielding SVR in nearly all patients. However, more epidemiological research is needed regarding the effect of SVR achievement on the development of T2DM. More importantly, DAA therapy leads to both better fasting glucose and HbA1C controls in patients with T2DM, and with prediabetes most likely also. Reducing antidiabetic treatment in some of the patients is possible. According to conclusions of the preliminary studies, DAA therapy improves hypertension control and atherosclerotic plaques. Furthermore, DAA therapy alternates lipoprotein profile considerably. Further research, however, is needed to evaluate its clinical significance. Most likely, DAA treatment and subsequently SVR achievement decrease cardiovascular risk. This fact is another reason for early treatment of patients, including those with a lower grade of liver fibrosis. Yet, chronic hepatitis C treatment remains inaccessible not only in

Table 2: Studies reporting the changes of lipoprotein metabolism after DAA treatment.

Author	Country	Genotype	Treatment	Increase of total cholesterol during or after DAA treatment	Increase of LDL-C during or after DAA treatment	Increase of HDL- C during or after DAA treatment	Decrease of TG during or after DAA treatment
Sun [67]	Taiwan	Genotype 1	Sofosbuvir + ledipasvir or Grazoprevir + elbasvir	Yes	NA	NA	Yes
Meissner [56]	USA	Genotype 1	Sofosbuvir + ribavirin	NA	Yes	NA	Yes
Chida [68]	Japan	Genotype 1b	Daclatasvir + asunaprevir	Yes	Yes	Yes	NA
Endo [69]	Japan	Genotype 1b	Sofosbuvir + ledipasvir or daclatasvir + asunaprevir	Yes	Yes	Yes	NA
Inoue [70]	Japan	Genotype 1b	Sofosbuvir + ledipasvir or Sofosbuvir + ribavirin or daclatasvir + asunaprevir	Yes	Yes	NA	Yes
Chaudhury [66]	USA	Genotype 1	Multiple DAA	Yes	Yes	NA	Yes
Hashimoto [71]	Japan	Genotype 1	Sofosbuvir + ledipasvir or daclatasvir + asunaprevir	NA	Yes	NA	NA
Meissner [56]	USA	Genotype 1	Sofosbuvir + ribavirin	Yes	Yes	NA	NA
Townsend [72]	USA	Genotype 1	Mostly sofosbuvir based	Yes	Yes	Yes	No
Beig [65]	New Zealand	Mostly genotype 1	Mostly sofosbuvir based	Yes	Yes	No	No
Carvalho [73]	Portugal	Mostly genotype 1	Mostly sofosbuvir based	Yes	Yes	No	No
Gitto [74]	Italy	Mostly genotype 1	Mostly sofosbuvir based	Yes	Yes	NA	NA
Mauss [75]	Germany	Mostly genotype 1	Multiple DAA	Yes	Yes	NA	NA
Morales [58]	USA	Mostly genotype 1	Only sofosbuvir based	Yes	Yes	NA	NA
El Sagher [64]	Egypt	Genotype 4	Sofosbuvir + simeprevir	Yes	Yes	Yes	NA
Tran [54]	Multi- ethnic	Genotype 1	Paritaprevir/ ritonavir + dasabuvir + ombitasvir	NA	NA	NA	Yes, also in patients with baseline elevated TG

NA: not available, LDL-c: low density lipoproteins, HDL-c: high density lipoproteins, and TG: triglycerides.

developing countries but also in countries with high quality of life [83].

Conflicts of Interest

Sylvia Drazilova reports personal fees and nonfinancial support from AbbVie, Gilead, MSD, outside the submitted work. Martin Janicko reports personal fees and nonfinancial support from AbbVie and nonfinancial support from Gilead, outside the submitted work. Peter Jarcuska reports personal fees and nonfinancial support from AbbVie and Gilead and personal fees from MSD, outside the submitted work. Jakub Gazda reports no conflicts of interest.

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Research Article

Predictive Role of Interleukin-18 in Liver Steatosis in Obese Children

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Introduction. Interleukin-18 (IL-18) is a proinflammatory cytokine associated with metabolic syndrome (MS). Nonalcoholic fatty liver disease (NAFLD) can be recognized as a feature of MS. *Material and Methods*. Serum IL-18 concentration was evaluated in serum of 108 obese children, determined with ELISA, and referred to degree of liver steatosis in USG or total intrahepatic lipid content assessed by magnetic resonance proton spectroscopy (^{1}HMRS). *Results*. Fatty liver was confirmed in 89 children with USG and in 72 with ^{1}HMRS. IL-18 concentration demonstrated significantly higher values in patients than in controls. Significant correlations between IL-18 and ALT, GGT, triglycerides, hsCRP, and the degree of liver steatosis were demonstrated. NAFLD children had significantly higher level of IL-18, ALT, GGT, HOMA-IR, waist circumference, and total lipids content in ^{1}HMRS than other obese children. IL-18 level was also significantly higher in obese children with advanced liver steatosis. Measurement of serum IL-18 showed ability to differentiate children with fatty liver from those without steatosis. *Conclusion*. Elevated serum IL-18 concentration and its correlation with hepatocyte injury, systemic inflammation, and degree of liver steatosis support role in NAFLD pathomechanism. IL-18 can be considered to play a role in predicting advanced liver steatosis and fatty liver in obese children.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) nowadays becomes a leading cause of liver pathology and may be present in 20–30% of general population. Rising prevalence of NAFLD is also observed among children and adolescents, which is closely related to obesity epidemic worldwide [1, 2]. The histological spectrum of NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), where steatosis coexists with hepatic inflammation that can be accompanied by fibrosis. Long lasting NASH may progress to severe liver damage and can result in cirrhosis or even hepatocellular carcinoma [3, 4]. Development of NAFLD is strongly associated with visceral obesity and other components of the metabolic syndrome (MS) including dyslipidemia and insulin resistance (IR); that is why NAFLD is regarded as a hepatic manifestation of MS [5, 6]. The pathogenesis of this clinical condition has not been fully understood but usually is explained by a "two-hit" hypothesis [7, 8]. In this mechanism the first "hit" is associated with excess accumulation of lipids in the liver. The second "hit" can lead to progression to NASH through increase of oxidative stress, lipid peroxidation, imbalance of adipokines level, or release of toxic products. Recent studies have revealed more possible factors involved in the development and progression of NAFLD, such as dysregulated intestinal microbiota, mitochondrial dysfunction, abnormalities of iron metabolism, or increased fructose consumption [9–15]. Moreover there are growing evidences showing that NAFLD, as well as IR, is strongly related to low-grade systemic inflammation and probably therefore some

mediators are released from obese fat tissue and additionally promoting hepatic and systemic inflammation [16–21]. For this reason we have focused on investigation of proinflammatory cytokine interleukin-18 (IL-18) and its possible association with the development and the potential progression of NAFLD through stimulation of hepatic inflammation and fibrosis. However the most recent studies demonstrate differences between pediatric and adult NAFLD in terms of prevalence, histology, diagnosis, and management [22].

IL-18 plays a crucial role in the inflammatory cascade. This molecule is a member of the IL-1 family of cytokines and it was initially described as an interferon-γ- (IFN-gamma-) inducing factor. IL-18 is secreted in many different cell types, including macrophages, endothelial cells, vascular smooth muscle cells, dendritic cells, Kupffer cells, and adipocytes [23, 24]. It is primarily synthesized as a precursor protein, pro-IL-18, which requires activation by caspase-1 cleavage into bioactive mature form. IL-18 is inactivated by specific binding protein, which results from a negative feedback mechanism in response to increased IL-18 production, protecting cells from accelerated proinflammatory activity [25, 26]. Previously published studies demonstrated elevated circulating levels of IL-18 in adult patients with metabolic syndrome and its components. There are several reports suggesting its usefulness as a marker of insulin resistance in type 2 diabetes and predictor of cardiovascular disease development [27–30]. Furthermore there is some evidence that plasma levels of IL-18 are elevated in adults with chronic liver disease and correlate with severity of the disease [31–33].

These findings were demonstrated in adults and led us to investigate whether there is an association between serum IL-18 concentration and NAFLD in obese children. Moreover we analyzed effect of steatosis degree on serum IL-18 levels to establish its possible predictive role for the disease progression in comparison to several other markers of hepatic injury, metabolic dysfunction, and systemic inflammation.

2. Materials and Methods

2.1. Patients. This study comprised a group of 108 consecutive obese children (84 boys and 24 girls) aged 7–17 years (mean age 12,8 years) admitted to our department because of suspected liver disease based on hepatomegaly and/or elevated alanine aminotransferase (ALT) activity and/or fatty liver features on ultrasound (USG) examination. Informed consent was obtained from all patients' parents. The protocol was approved by the local bioethics committee. Viral infection due to hepatitis C virus (negative testing for anti-HCV) and hepatitis B virus (negative testing for HBsAg), autoimmune hepatitis, selected metabolic liver diseases (Wilson disease, alpha-1-antitrypsin deficiency, and cystic fibrosis), and drug-induced liver injury (DILI) were excluded in all children. Moreover, children with diabetes (elevated fasting plasma glucose) and those having infectious diseases on admission (white blood cell (WBC) count less than 3500 cells/μl or more than 10 500 cells/μl; C-reactive protein (CRP) more than 5 mg/L; body temperature more than 38°C) were excluded from this study.

All participants underwent a detailed medical history and physical examination with anthropometric measurements, including weight, height, body mass index (BMI), and waist circumference. BMI was calculated by dividing weight in kilograms by the square of height in meters (kg/m2). Obesity was defined as a BMI over the 95th percentile based on age and gender [34]. The diagnosis of NAFLD was established in children with both elevated serum ALT activity and liver steatosis on ultrasound examination. Liver steatosis was diagnosed with positive imaging results. Patients were divided into two groups: with NAFLD ($n = 39$) and obese without NAFLD ($n = 69$) that did not fulfil criteria of NAFLD diagnosis. All patients with elevated ALT had steatosis, but there were 50 patients with steatosis and normal ALT.

Age matched control group comprised of 15 nonobese children without any somatic organ pathology was recruited at the time of the study and blood samples were collected as a part of their routine checkup to obtain normal values of IL-18.

2.2. Laboratory Measurements. In all patients routine biochemical measurements were performed, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), bilirubin, total cholesterol, high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) cholesterol, triglycerides (TG), uric acid, ferritin, glucose, and insulin, by using the enzymatic colorimetric method at laboratory department of our hospital. In all patients alpha-1-antitrypsin (A1AT) level and in 45 patients high-sensitive C-reactive protein (hsCRP) level were determined by a nephelometric method with a DADE Behring Nephelometer Analyzer (Germany).

Insulin resistance (IR) was calculated by homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: HOMA-IR = fasting insulin (μU/ml) × fasting plasma glucose (mmol/L)/22.5 [35].

Serum IL-18 levels were determined by an enzyme-linked immunosorbent assay (Human IL-18 ELISA Kit, Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Samples were centrifuged and frozen in −80°C until further analysis. The minimum detection limit for IL-18 was 12.5 pg/ml. The intra- and interassay CVs of IL-18 ranged from 4.9 to 10.8% and 5.2 to 10.1%, respectively. The assays were conducted according to the manufacturer's instructions.

2.3. Liver Examination. Liver ultrasound examination was performed with Voluson E8 instrument (General Electric, USA), equipped with convex sonde 3–5 MHz. The degree of liver steatosis was graded using a four-grade scale (0–3) according to Saverymuttu et al. [36]. Advanced liver steatosis was defined as a score > 1. Steatosis grade was assessed by the same radiologist without knowledge of the patients' data. Proton magnetic resonance spectroscopy (^{1}HMRS) was performed with a 1.5 T scanner (Picker Eclipse) and with PRESS sequencing. Total intrahepatic lipid content was assessed in relative units (r.u.) in comparison to unsuppressed water signal. Voxel's size 3 × 3 × 3 cm (27 cm3) was localized in the right liver lobe to avoid vessels and bile ducts [37].

Table 1: Analyzed parameters in all obese patients included in the study.

Parameters	NAFLD median; Q1–Q3 ($n = 39$)	Non-NAFLD median; Q1–Q3 ($n = 69$)	p
Gender, M/F	33/6	51/18	NS
IL-18 (pg/ml)	335; 257–493	299; 219–383	0.034
BMI (kg/m^2)	29; 27–34	27; 25–31	0.007
Waist (cm)	100; 94–108	93; 86–103	0.008
ALT (IU/l)	64; 50–101	26; 19–33	0.000
AST (IU/l)	39; 32–52	25; 21–30	0.000
GGT (IU/l)	30; 23–45	18; 14–23	0.000
Bilirubin (mg/dl)	0.6; 0.5–0.9	0.6; 0.45–0.7	NS
Total cholesterol (mg/dl)	177; 153–216	174; 145–190	NS
HDL-C (mg/dl)	44; 39–51	46; 38–54	NS
LDL-C (mg/dl)	97; 79–143	99; 78–116	NS
TG (mg/dl)	132; 85–173	108; 85–142	NS
Glucose (mg/dl)	93; 85–99	91; 86–96	NS
Insulin (μIU/ml)	18; 14–22	15; 11–18	0.011
HOMA-IR	4.2; 3.2–5.8	3.2; 2.1–4	0.005
Uric acid (mg/dl)	6.5; 5.5–7.4	5.7; 4.8–6.6	0.023
hsCRP (mg/l)	0.9; 0.7–1.8	1.3; 0.6–2	NS
Ferritin (ng/ml)	61; 50–105	52; 34–65	0.005
A1AT (g/l)	1.3; 1.2–1.5	1.4; 1.2–1.5	NS
USG grade of steatosis	2; 1–3	1; 0-1	0.000
^{1}HMRS (r.u.)	163; 106–210	80; 40–148	0.000

2.4. Statistical Analysis. Results are reported as median, 25–75 quartiles. The statistical analysis was evaluated using the Mann–Whitney two-sample test for nonparametric data. The relationship between serum IL-18 concentration and variables was analyzed by the Spearman rank-correlation test for nonparametric data. Statistical significance level was set at $p < 0.05$. Logistic regression analysis was performed using IBM SPSS Statistics 20.0. The receiver operating characteristics (ROC) analysis was used to calculate the power of IL-18 to detect liver steatosis on ^{1}HMRS. The comparison of the area under curve (AUC) was performed using a two-tailed p-test, which compares the AUC to the diagonal line of no information (AUC 0.5).

To perform combined ROC analysis of multiple parameters, linear combination of logistic regressions was carried out to detect children with liver steatosis in ^{1}HMRS.

3. Results

Eighty-nine children (82,5%) had liver steatosis in ultrasound examination and 72 (78,2%) in ^{1}HMRS with concordance rate of 100%. Moreover thirty-nine had also an elevated serum ALT activity (NAFLD patients).

Median IL-18 concentration measured in all obese patients (309; 237–410 pg/mL) was significantly ($p = 0.038$) higher than in controls (242; 197–318 pg/mL). As demonstrated in Table 1 median IL-18 level was significantly higher in NAFLD patients than in other obese children ($p = 0.034$). Moreover, patients with NAFLD showed significantly higher values of ALT, AST, and GGT activities, insulin, HOMA-IR, uric acid, ferritin, BMI, waist circumferences, degree of liver steatosis (USG), and total amount of lipids in ^{1}HMRS compared to children without NAFLD (Table 1).

Significant positive correlations of IL-18 with ALT ($r = 0.2$, $p = 0.036$), AST ($r = 0.21$, $p = 0.032$), GGT ($r = 0.23$, $p = 0.016$), TG ($r = 0.21$, $p = 0.027$), hsCRP ($r = 0.36$, $p = 0.014$), and the degree of liver steatosis (USG) ($r = 0.2$, $p = 0.044$) were found (Table 2). Patients with steatosis in USG and elevated ALT demonstrated significantly higher IL-18 activity than those with steatosis but normal ALT (310 versus 339 pg/mL; $p = 0.044$). However it was not a case in patients with steatosis diagnosed using ^{1}HMRS (341 versus 349 pg/mL; p-0.064).

Among 108 obese children included in the study, 19 had no steatosis in USG (grade 0), 47 (44%) demonstrated moderate steatosis (grade 1), and 42 (39%) had advanced liver steatosis defined as grade 2 or 3 (Table 3). The concentration of IL-18 was significantly higher in obese children with advanced liver steatosis compared to children without steatosis ($p = 0.027$) (Figure 1).

As shown in Table 4 children with liver steatosis measured with ^{1}HMRS had significantly higher levels of IL-18, ALT, AST, GGT, TG, and intensity of liver steatosis in USG examination than those without liver steatosis in ^{1}HMRS.

Table 2: Correlation (Spearman) between IL-18 serum concentrations and selected parameters.

	r	p
ALT	0.20	**0.036**
AST	0.21	**0.032**
GGT	0.23	**0.016**
Bilirubin	−0.13	0.185
Total cholesterol	−0.02	0.811
HDL-C	−0.08	0.399
LDL-C	−0.02	0.841
TG	0.21	**0.027**
Glucose	0.11	0.253
Insulin	0.07	0.462
HOMA - IR	0.08	0.396
Uric acid	−0.04	0.704
hsCRP	0.36	**0.014**
Ferritin	0.05	0.599
A1AT	−0.05	0.588
USG grade of steatosis	0.19	**0.044**
1HMRS	0.12	0.252
BMI	−0.12	0.219
Waist	−0.29	**0.009**

In the logistic regression analysis IL-18 (OR-1.006, 95% CI for OR—1.001–1.010, and $p = 0.018$) and triglycerides (OR-1.015, 95% CI for OR—1.003–1.028, and $p = 0.012$) were the only measures useful in the differentiation of obese patients with liver steatosis confirmed using ^{1}HMRS. This finding was confirmed in ROC analysis, which demonstrated a cut-off of IL-18 concentration on the level of 326.8 pg/ml as effective (AUC = 0.68; $p = 0.006$) for differentiation between children with or without fatty liver in ^{1}HMRS (Table 5). Sensitivity was 60% and specificity 75%, whereas negative and positive predictive values were 34% and 90%, respectively (Figure 2(a)). ROC curves for ALT, AST, GGT, and TG demonstrate similar pattern to IL-18 (Figures 2(b), 2(c), 2(d), and 2(e)) as well as AUC values (Table 5). Combined ROC analysis of all five parameters was carried out to find out possible superior measure for steatosis corresponding to intrahepatic lipid content evaluated with ^{1}HMRS. ROC analysis of combined parameters demonstrated superior AUC of 0.7826 ($p < 0.0001$), sensitivity 61%, specificity 85%, and negative or positive predictive value of 38% and 94%, respectively. However the ROC of the combined logistic regression model did not demonstrate statistical significance compared to individual AUC (Figure 2(f), Table 5).

4. Discussion

Early diagnosis of NAFLD in obese children is important for possible prediction of further metabolic disorders, which was supported in our study through significantly higher insulin concentration and HOMA-IR values related to insulin resistance. In this group of obese children with diagnosed NAFLD, we demonstrated significantly higher IL-18 serum concentration compared to both healthy controls and non-NAFLD obese children. This is in line with results from the study of Vecchiet at al. [33] who demonstrated higher IL-18 in NAFLD adult patients compared to controls but lower IL-18 than in patients with chronic hepatitis C, indicating possible role of hepatic inflammation in addition to steatosis regarding IL-18 production. The significant positive correlations between serum IL-18 and ALT and other indices of hepatocytes injury as well as hsCRP shown in our study support this hypothesis. However, we were not able to demonstrate significant elevation of hsCRP in any group, because its measurement was carried out in limited number of patients.

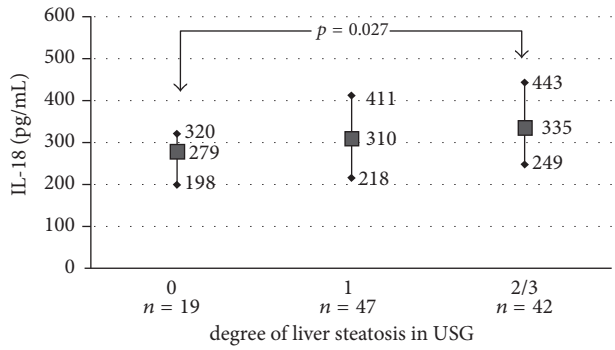

Figure 1: Median (min., max.) of IL-18 serum concentration depending on degree of liver steatosis in USG.

Contrary results with no effect on IL-18 in the absence of metabolic risk factors were demonstrated by Tapan et al. [38], but the study was conducted in male adults with NASH. IL-18 is a proinflammatory cytokine that can be produced by adipocytes, but also macrophages and Kupffer and endothelial cells [23, 24]. Therefore serum IL-18 production can be released as a result of inflammatory activity related to numerous concomitant health conditions, which obviously are much more frequent in adults than in children. Since children are less likely to have concomitant proinflammatory conditions than adults, measurement of IL-18 concentration can be useful for NAFLD diagnosis in children. In this study we were able to demonstrate significant increase of IL-18 serum concentrations with degree of liver steatosis measured using USG. To support results obtained with USG we applied ^{1}HMRS. Obtained results demonstrated that hepatopathic children with liver steatosis measured with ^{1}HMRS have significantly higher levels of serum IL-18 as well as steatosis score of 2 or 3 in USG than those without liver steatosis in ^{1}HMRS. IL-18 serum level above 326.8 pg/mL was finally demonstrated with ROC analysis as an optimal border for differentiation of NAFLD in children. Interestingly elevated IL-18 was accompanied by higher activities of hepatocyte injury enzymes in more advanced steatosis irrespective of diagnostic method with USG or ^{1}HMRS. On the other hand there was no effect of steatosis degree on cholesterol and glucose levels, but possible future metabolic problems in children with more advanced steatosis were indicated by significantly higher triglycerides level and insulin resistance.

Table 3: Comparison of obese children without liver steatosis ($n = 19$) and with advanced liver steatosis ($n = 42$) evaluated in USG according to Saverymuttu et al.

Parameters	No liver steatosis USG grade 0 median; Q1–Q3 ($n = 19$)	Advanced liver steatosis USG grade 2-3 median; Q1–Q3 ($n = 42$)	p
Gender, M/F	12/7	36/6	NS
IL-18 (pg/ml)	279; 198–320	335; 249–443	0.027
BMI (kg/m^2)	27; 25–30	28; 27–33	NS
Waist (cm)	90; 83–98	99; 93–107	0.014
ALT (IU/l)	20; 16–31	48; 36–77	0.000
AST (IU/l)	23; 20–29	32; 26–45	0.000
GGT (IU/l)	18; 13–23	27; 21–35	0.000
Bilirubin (mg/dl)	0.6; 0.5–0.7	0.6; 0.4–0.85	NS
Total cholesterol (mg/dl)	164; 137–182	170; 153–191	NS
HDL-C (mg/dl)	47; 39–56	42; 37–50	NS
LDL-C (mg/dl)	92; 73–111	96; 79–121	NS
TG (mg/dl)	86; 65–116	120; 88–167	0.004
Glucose (mg/dl)	90; 83–96	95; 90–99	NS
Insulin (μIU/ml)	14; 8–17	17; 12–21	NS
HOMA-IR	3.2; 1.9–3.7	3.9; 3–5.4	0.020
Uric acid (mg/dl)	5.9; 4.8–7	6.7; 5.6–7.3	NS
hsCRP (mg/l)	1.1; 0.7–2.2	1.1; 0.5–1.7	NS
Ferritin (ng/ml)	54; 29–66	58; 46–85	NS
A1AT (g/l)	1.4; 1.2–1.6	1.3; 1.2–1.4	NS
^{1}HMRS (r.u.)	38; 17–61	172; 123–216	0.000

Table 4: Comparison of obese children with ($n = 72$) and without ($n = 20$) liver steatosis diagnosed with measurement of intrahepatic lipid content using ^{1}HMRS.

Parameters	No liver steatosis in ^{1}HMRS median; Q1–Q3 ($n = 20$)	Liver steatosis in ^{1}HMRS median; Q1–Q3 ($n = 72$)	p
Gender, M/F	12/8	58/14	NS
IL-18 (pg/ml)	275; 205–340	342; 249–455	0.014
BMI (kg/m^2)	29; 27–32	28; 26–32	NS
Waist (cm)	96; 88–104	94; 90–105	NS
ALT (IU/l)	25; 16–45	37; 26–63	0.022
AST (IU/l)	24; 21–33	30; 25–39	0.013
GGT (IU/l)	17; 12–23	23; 18–31	0.016
Bilirubin (mg/dl)	0.7; 0.5–1.2	0.6; 0.4–0.8	NS
Total cholesterol (mg/dl)	172; 142–182	176; 150–192	NS
HDL-C (mg/dl)	50; 42–52	46; 39–55	NS
LDL-C (mg/dl)	92; 78–114	97; 78–121	NS
TG (mg/dl)	90; 61–120	115; 88–164	0.008
Glucose (mg/dl)	90; 85–95	92; 85–96	NS
Insulin (μIU/ml)	14; 8–18	15; 12–20	NS
HOMA-IR	3; 1.9–4	3.6; 2.8–4.6	NS
Uric acid (mg/dl)	5.7; 4.8–7	6; 5–6.8	NS
hsCRP (mg/l)	1; 0.7–2	1.3; 0.6–1.9	NS
Ferritin (ng/ml)	55; 29–73	57; 45–86	NS
A1AT (g/l)	1.4; 1.2–1.6	1.3; 1.2–1.5	NS
USG grade of steatosis	0.5; 0-1	1.5; 1-2	0.000

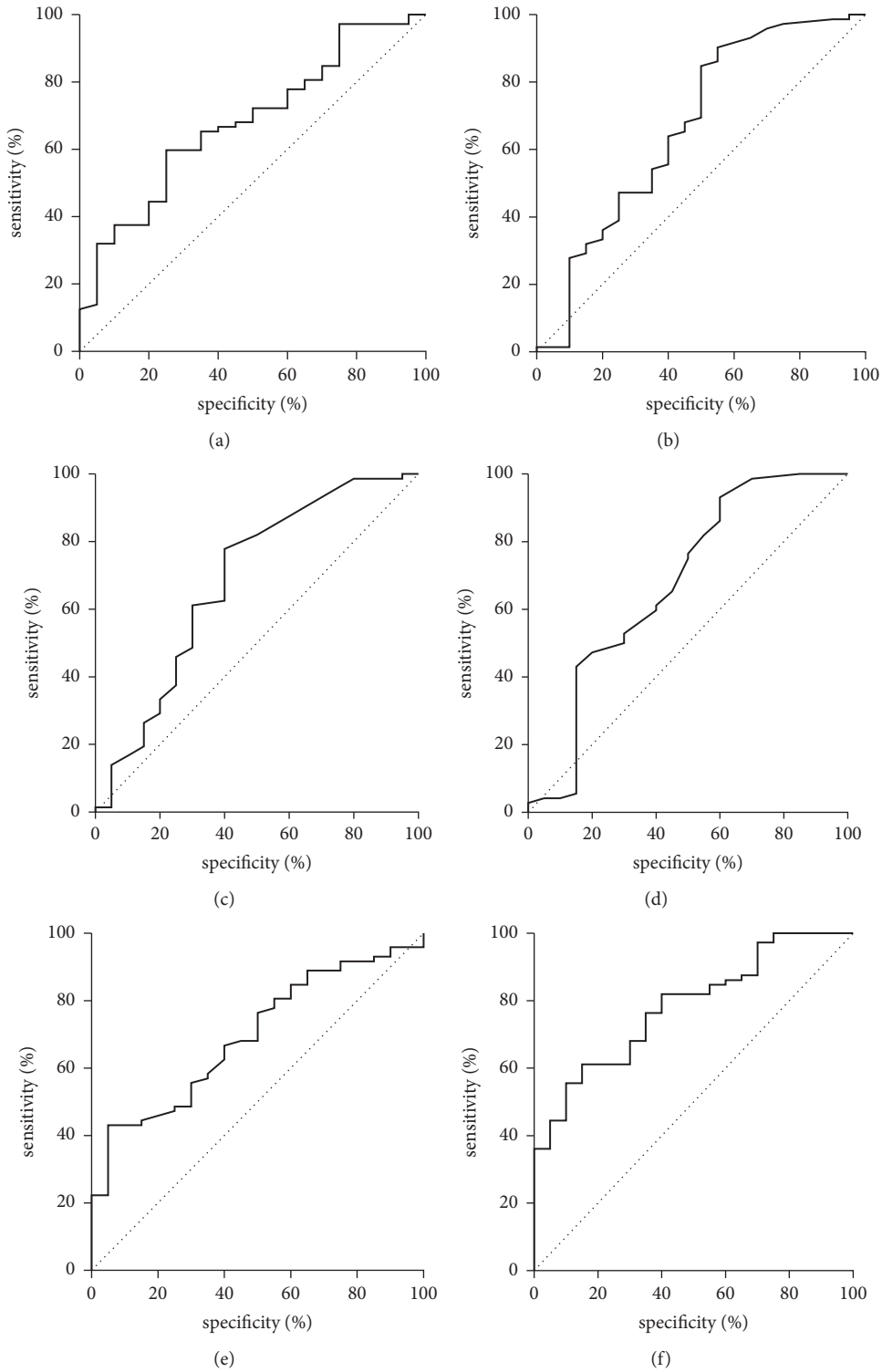

Figure 2: ROC curve for ability of IL-18 (a), ALT (b), AST (c), GGT (d), TG (e), and their combination (f) to detect children with liver steatosis in [1]HMRS.

Table 5: Analysis of AUC for IL-18, ALT, AST, GGT, and TG and their combination to detect children with liver steatosis in ^{1}HMRS.

Parameters	AUC	SE	95% CI (AUC)	p (AUC = 0,5)	p (AUC individual versus combined)
IL-18	0,6802	0,0653	(0,552–0,808)	0,0058	0,263
ALT	0,6681	0,0787	(0,514–0,822)	0,0327	0,253
AST	0,6830	0,0769	(0,532–0,834)	0,0173	0,279
GGT	0,6767	0,0792	(0,521–0,832)	0,0257	0,324
TG	0,6944	0,0614	(0,574–0,815)	0,0015	0,316
Combined	0,7826	0,0532	(0,678–0,887)	<0,0001	-

The studies on adults population have suggested that iron overload plays a significant role in pathogenesis of NAFLD and progression from simple steatosis to nonalcoholic steatohepatitis (NASH) through increasing oxidative stress and altering insulin signaling and lipid metabolism [13]. Since serum ferritin level is related to liver iron storage in NAFLD [39], we included measurement of ferritin in our study. Kowdley at al. [40] demonstrated recently elevated serum ferritin as an independent predictor of advanced hepatic fibrosis among adult patients with NAFLD. Our data confirmed possibility of similar effect in younger population, because of significantly higher ferritin serum concentration among obese NAFLD children. However, in contrast to IL-18 increase of ferritin serum level was not related to liver steatosis, which is contrary to data from adult population that showed predictive role of high serum ferritin as a risk factor for steatosis [41]. Based on these data we can conclude that serum ferritin is related mostly to inflammatory activity in NAFLD patients [42]. However according to results from our study and some literature data, this component of the disease can also be measured using hsCRP [43]. In contrast, elevated IL-18 can reflect degree of steatosis in addition to inflammation and therefore can be recognized as more representative for complexity of NAFLD pathogenesis.

This is a first study in obese children population investigating serum IL-18 concentration. Studies carried out in children may be more reliable compared to those in adults, because of infrequent confounding factors and less advanced disease. Large sample size of obese children is a main strength of our study. Additional value of our study was inclusion of new sensitive imaging technique to assess liver steatosis, such as proton magnetic resonance spectroscopy (^{1}HMRS), providing an opportunity of precise data interpretation [44]. However there are some potential limitations of the study. First of all, we did not perform histological examination to confirm diagnosis in the studied group. According to the ESPGHAN Hepatology Committee guidelines liver biopsy is preferred but imperfect gold standard, because as an invasive procedure it has important limitations in children, including risk of complications, cost, and possible sampling error [45]. Therefore, according to accepted recommendations liver biopsy should be performed only in children in very specific cases of NAFLD suspicion, such as suspected advanced disease, to exclude coexisting diseases, in patients below 10 years of age with elevated ALT activity and before therapeutic intervention [46]. Children included in our study did not meet these criteria; therefore we did not perform a liver biopsy. We still need to validate reliable, noninvasive tests that could be useful to evaluate children with suspected NAFLD and determine disease progression. Another limitation of our study was selection bias, because examined patients were recruited from a tertiary center, which is focused on pediatric hepatology. Therefore children with suspected liver disease were initially referred to the center and then included in the study group.

In conclusion, the study demonstrated association between NAFLD and IL-18 serum concentration in children. Based on this finding we can consider IL-18 as a useful novel noninvasive biomarker for differential diagnosis between obese children with and without NAFLD and prediction of possible metabolic disorders development.

Abbreviations

ALT: Alanine aminotransferase
A1AT: Alpha-1-antitrypsin
AUC: Area under curve
AST: Aspartate aminotransferase
BMI: Body mass index
CRP: C-reactive protein
DILI: Drug-induced liver injury
GGT: Gamma glutamyltransferase
HBV: Hepatitis B virus
HCV: Hepatitis C virus
HDL: High-density lipoprotein
hsCRP: High-sensitive C-reactive protein
HOMA-IR: Homeostasis model assessment of insulin resistance
IR: Insulin resistance
IFN-gamma: Interferon-γ
IL-18: Interleukin-18
LDL: Low-density lipoprotein
MS: Metabolic syndrome
NAFLD: Nonalcoholic fatty liver disease
NASH: Nonalcoholic steatohepatitis
^{1}HMRS: Proton magnetic resonance spectroscopy
ROS: Reactive oxygen species
ROC: Receiver operating characteristics
TG: Triglycerides
USG: Ultrasonography
WBC: White blood cell.

Ethical Approval

The study was approved by the bioethics committee of the Medical University of Białystok. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent

Consent was obtained from all individual participants included in the study.

Conflicts of Interest

The authors have no conflicts of interest.

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