

Evaluation of metabolic syndrome in patients with non-alcoholic steatohepatitis

¹Nicoleta V. Leach, ²Eleonora Dronca, ³Corina Radu, ³Dana Crisan, ⁴Monica Lupsor-Platon, ¹Dorel P. Sampelean, ³Mircea Grigorescu

¹Vth Department of Internal Medicine, “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania; ²Department of Medical Genetics, Faculty of Medicine, “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania; ³Regional Institute of Gastroenterology and Hepatology, “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania; ⁴Medical Imaging Department, “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Abstract. Introduction: Non-alcoholic fatty liver disease (NAFLD) is closely associated with abdominal obesity, hypertension, dyslipidemia and type 2 diabetes, which are all features of metabolic syndrome (MS), a highly pro-atherogenic condition. Aim: Our study aimed to evaluate the prevalence of MS and its components in patients with non-alcoholic steatohepatitis (NASH). Patients and methods: 50 patients with non-alcoholic steatohepatitis and 30 healthy controls, age and gender matched, were recruited. The diagnosis of MS was made according to AHA/NHLBI 2009 consensus criteria. Lipid profile, liver biochemical markers, fasting plasma glucose (FPG), insulin level, insulin resistance index (HOMA-IR) and visceral fat thickness (VFT) were assayed. Results: The prevalence of MS in NASH patients was 53.1%. Abdominal obesity had the highest prevalence (96.1%), followed by hypertension (92%) and decreased HDL-cholesterol (87.1%). IR prevalence was of 69.7% in NASH patients and of 80.8% in patients with NASH and MS. HOMA-IR was positively correlated with abdominal obesity and transaminase levels. VFT was the only factor independently associated with IR. VFT increase was positively correlated with body mass index (BMI), waist circumference (WC), IR, transaminase levels, total cholesterol, LDL-cholesterol, triglycerides (TG) and negatively correlated with HDL-cholesterol. High BMI was the only factor independently associated with increased VFT. Conclusion: Patients with NASH showed a marked increase in the prevalence of MS compared to the general population. Abdominal obesity plays a central role in the development of IR, MS and liver inflammation. Ultrasound measurement of VFT is proving to be a very good independent predictor of NASH, both in women and men and, also, a simple and non-invasive ultrasound method for assessing abdominal obesity.

Key Words: non-alcoholic steatohepatitis, metabolic syndrome, insulin resistance, visceral fat thickness.

Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Corresponding Author: N. V. Leach, email: nicoleta.leach@umfcluj.com

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents the commonest liver disorder in clinical practice, comprising a series of conditions, ranging from simple steatosis to steatohepatitis (NASH) which can progress to cirrhosis, in the absence of significant alcohol consumption (<20 g/day for women and <30 g/day for men) (Ratziu et al 2009, Radu et al 2008, Brunt et al 2005). NAFLD has continually increasing incidence and prevalence in both adult and pediatric population due to the growing epidemic of obesity and type 2 diabetes (Angulo et al 2002). Numerous studies support the idea that NAFLD is the hepatic manifestation of MS. It covers a range of diseases (i.e. central obesity, hypertension, type 2 diabetes, hypertriglyceridemia, decreased HDL-cholesterol) that share the presence of insulin resistance (IR) as a pathogenic mechanism.

The presence of metabolic syndrome (MS) in patients with NASH is accompanied by the increase severity of liver disease and increased cardiovascular risk (Kotronen et al 2008). Abdominal obesity and IR are the most important pathogenic factors in NASH, MS and atherosclerosis (ATS) (Mottilo et al 2010). The mechanisms by which central obesity is involved

in ATS onset and progression are incompletely understood. Increased abdominal obesity is associated with presence of traditional cardiovascular risk factors and it is the most common feature of MS (Finelli et al 2013).

Central obesity is associated with increased cardiovascular morbidity and mortality independent of other cardiovascular risk factors (Kuk et al 2006).

Visceral adipose tissue (VAT) is not only an adipose tissue storage but it is also an endocrine organ able to secrete a number of molecular mediators, such as adipokines (adiponectin, leptin, resistin), cytokines (TNF, IL-6, etc), chemokines (MCP-1), free fatty acids (FFA), which reach the liver through the portal vein and contribute to the occurrence of IR and liver inflammation. Compared to subcutaneous fat, visceral adiposity contains the largest number of hypertrophied adipocytes and inflammatory cells, so it is more insulin resistant and has a higher lipolysis rate (Finelli et al 2013). Ultrasound VFT measurement is considered a reliable indicator of central obesity. VFT is considered the best predictor for IR and cardiovascular disease (Freedland et al 2004).

The purpose of this study was to evaluate the prevalence of MS and its components in patients with NASH.

Patients and methods

Study Participants

The study group comprised 50 patients previously diagnosed with NASH through liver biopsy NAFLD activity score ≥ 3) (Kleiner *et al* 2005) and reconfirmed by abdominal ultrasonography (and 30 healthy subjects of similar age (45.7 ± 10.9 vs. 44.9 ± 7.7 years) and sex (males/females 35/15 vs. 20/10). Other known etiologic factors for chronic liver disease (i.e. alcohol intake >30 g for men and >20 g for women, viral or autoimmune hepatitis, primary biliary cirrhosis, Wilson's disease, α 1-antitrypsin deficiency, use of hepatotoxic drugs) were excluded. None of patients had kidney disease, liver cirrhosis or carcinoma or other cancers.

For the control group, inclusion criteria were normal liver ultrasound, negative serology for viral hepatitis and normal liver biochemical tests.

The study protocol was designed in accordance with the Declaration of Helsinki and was approved by the University Ethics Committee.

All participants to the study signed an informed written consent. All subjects underwent a complete clinical, laboratory and ultrasound evaluation.

Clinical evaluation

Personal history and detailed physical examination were evaluated for all participants, using a questionnaire including personal data, demographic and anthropometric data [weight, height, waist circumference (WC), waist/hip ratio (WHR)], cardiovascular risk factors (e.g. smoking, sedentary lifestyle, history of diabetes, dyslipidemia and high blood pressure) and daily alcohol consumption.

Weight (expressed in kg) was measured twice, to the nearest 0.05 kg (the average was used) using an electronic scale with subjects without shoes and wearing only light indoor clothing. Height (expressed in cm) was measured twice, to the nearest 0.1 cm (the average was used) with subjects in orthostatic position using a stadiometer. WC was measured in a standing position at the midpoint between the lower rib cage border and the iliac crest at the end of normal expiration, whereas hip circumference was (HC) was similarly obtained at the widest point between hips and buttocks, at the level of greater trochanters on both sides. The diagnosis of abdominal obesity implied a WC ≥ 80 cm in women and ≥ 94 cm in men (Alberti *et al* 2009). WHR was calculated as the WC to HC ratio; a WHR ratio ≥ 0.85 in women and ≥ 0.90 in men indicated the presence of android obesity.

Body mass index (BMI, kg/m^2) was calculated as the ratio of weight (in kilograms) to the square of height (in meters). BMI was normal if <25 kg/m^2 , overweight if between 25-30 kg/m^2 and obese if >30.1 kg/m^2 (WHO Obesity 2011). Sedentary lifestyle or physical inactivity was defined as less than five times of 30-minute moderate activity per week or less than three times of 20-minute vigorous activity per week, or equivalent. Unhealthy diet was considered as high dietary intakes of saturated fat, trans-fats and salt, low intake of fruits, vegetables and fish (WHF, 2011). The diagnosis of MS was based on the criteria proposed by the Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society;

and International Association for the Study of Obesity, in 2009. According to this definition, patients were classified as having MS if at least three of the following five criteria were present: (1) WC ≥ 94 cm in men or ≥ 80 cm in women, (2) triglycerides (TG) ≥ 150 mg/dl or fibrate treatment, (3) HDL-cholesterol ≤ 40 mg/dl in men and ≤ 50 mg/dl in women or cholesterol-lowering treatment, (4) fasting plasma glucose (FPG) ≥ 100 mg/dl or previously diagnosed type 2 diabetes mellitus, (5) blood pressure (BP) $\geq 130/85$ mm Hg or antihypertensive treatment (Alberti *et al* 2009).

Laboratory investigation

A blood sample was obtained in the morning, after a minimum 12 hour-overnight fasting. Common plasma biochemical markers [i.e. total cholesterol, HDL-cholesterol, LDL-cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl transpeptidase (GGT) and FPG] were assayed on an automatic analyzer (Konelab 301-Thermo Electron Corp., Finland) using standard laboratory procedures. Serum insulin antibodies were measured using an ELISA method, following the kit protocol (Diametra, Italy). The level of IR was calculated according to the homeostasis model assessment for insulin resistance (HOMA-IR) using the formula: FPG (mg/dl) x fasting serum insulin ($\mu\text{UI}/\text{ml}$)/405 (Matthews *et al* 1985). The cut-offs of ≥ 2 and ≥ 4 were chosen as criteria for IR and prediabetes, respectively (Romero- Gómez *et al* 2006).

Ultrasound evaluation

The same day of blood sampling, each participant underwent abdominal ultrasound investigation using a high resolution ultrasonographic system Logiq 7 (General Electric, USA) with a 5 MHz transducer, by a single trained operator who was blind to clinical characteristics and laboratory findings of participants. Hepatic steatosis was defined as the presence of diffuse hyper-echoic texture, bright liver, increased liver echo texture compared to the kidneys, vascular blurring and deep attenuation of the ultrasonic beam. The VFT and the subcutaneous fat thickness (SFT) were measured with the probe located 1 cm above the umbilicus on the xypho-umbilical line, in both longitudinal and transverse views, and defined as the distance between linea alba and the anterior wall of the aorta and as the distance between linea alba and the skin, respectively (Ramilli *et al* 2009; Sanyal *et al* 2002).

Statistical analysis

Data were grouped into nominal and quantitative variables. Nominal variables were expressed as frequencies and percentages. For evaluation of normal distribution of quantitative variables we used the Kolmogorov-Smirnov test; data were expressed as median and percentiles (25-75%), when they were normally distributed and as mean \pm standard deviation (SD), when they were not. To examine differences between the two groups in terms of a quantitative variable, we used the t-test for independent variables and the Mann-Whitney test, where appropriate. The correlation between two quantitative variables was performed with Pearson or Spearman's rho correlation, depending on the situation. Chi-square test was used to check for frequency differences of nominal variables between two groups. We used AUROC test to determine the threshold

Table 1. Clinical, demographic and biochemical features of the investigated groups

Variables	NASH group (n=50)	Control group (n=30)	p
Age (years)	45.76±10.9	44.9±7.79	0.7
Males (number; %)	35 (70%)	20 (66.7%)	0.8
Current smokers (%)	15 (30.6%)	3 (10%)	0.06
Sedentary lifestyle (number; %)	27 (55.1%)	7 (23.1%)	0.01
Unhealthy diet (number; %)	20 (40.8%)	7 (23.3%)	0.1
High blood pressure (number; %)	25 (50%)	0	<0.001
Diabetes mellitus (number; %)	5 (10.2%)	0	0.1
BMI (kg/m ²)	31.09±4.6	22.8±3.6	<0.001
WC (cm)	106.1±12.6	80.87±12.1	<0.001
WHR	1.02±0.09	0.85±0.08	<0.001
MS (%)	53.1%	0%	
Total cholesterol (mg/dL)	220.18±47	178.6±20.1	<0.001
HDL-cholesterol (mg/dL)	52.14±15.3	65.73±14.9	<0.001
LDL-cholesterol (mg/dL)	142.27±38.8	110.93±23.5	<0.001
TG (mg/dL)	130 (98; 267)	77 (56; 110)	0.002
FPG (mg/dL)	95 (88.2; 106)	89.5 (86; 95)	0.05
Insulin (μUI/mL)	11.86 (8.5; 18.9)	7.89 (5.9; 10.7)	0.004
HOMA-IR	2.81 (1.88; 4.87)	1.71 (1.35; 2.35)	0.001
HOMA-IR >2	30 (69.7%)	13 (43.3%)	0.04
AST (U/L)	38 (31; 56)	21 (17; 23)	<0.001
ALT (U/L)	55 (41; 78)	20 (17; 22)	<0.001
GGT (U/L)	59 (38; 77)	23 (18; 28)	<0.001
SFT (mm)	20.07±11.1	19.78±7	<0.001
VFT (mm)	87.51±21.1	50±16.1	<0.001

BMI-body mass index, WC-waist circumference, WHR-waist/hip ratio, MS-metabolic syndrome, TG-triglycerides, FPG-fasting plasma glucose, HOMA-IR-Homeostatic model assessment insulin resistance index, AST-aspartate aminotransferase, ALT-alanine aminotransferase, GGT-gamma glutamyl transpeptidase, SFT-subcutaneous fat thickness, VFT-visceral fat thickness, as mean±standard deviation (SD), median and range of 25-75% in parentheses

of a quantitative variable, predictive of a particular event and we calculated sensitivity and specificity of this threshold value. Multivariate analysis used multiple linear or binary logistic regression, depending on the situation.

A p value <0.05 was considered statistically significant.

For statistical analysis we used SPSS for Windows, version 21.0 (Chicago, Illinois, USA).

Results

The clinical, demographic and biochemical features of the investigated groups are shown in Table 1.

Age and gender distribution were similar in the NASH and control groups. Hypertension and sedentary lifestyle occurred more frequently in NASH patients. Half of NASH patients were hypertensive (19 patients had antihypertensive treatment) and five NASH patients had type 2 diabetes mellitus (none underwent insulin treatment).

In NASH patients, BMI, WC and WHR were significantly higher than in the control group. Obesity was present in 27 (54%)

of NASH patients, mean BMI values being 31.09±4.6 kg/m². Comparative analysis of obese patients with NASH showed that women accounted for 37.03% and had a BMI average of 34±4.2 kg/m² and men accounted for 72.97% and had a BMI average of 31±3.5 kg/m². BMI was significantly correlated with age (r=0.364; p=0.01), WC (r=0.751; p<0.001), SFT (r=0.321; p=0.01) and VFT (r=0.463; p=0.001).

As seen in Table 1, patients with NASH had all biochemical and ultrasound parameters significantly higher than the control group. Depending on the presence or absence of MS, the study group was divided into two groups that were compared according to clinical, biochemical and ultrasound parameters (Table 2).

In univariate analysis, factors significantly associated with MS in NASH patients were older age, hypertension, impaired FPG or diabetes, hypercholesterolemia, hypertriglyceridemia, IR, elevated WC, VFT, LDL-cholesterol values and low HDL-cholesterol values (Table 2).

In multivariate analysis, only HOMA-IR was an independent predictive factor for MS (OR 1.6 95% CI, 1-2.5; p=0.04).

Table 2. Characteristics of NASH patients based on the presence of the MS

Variable	NASH patients with MS	NASH patients without MS	P
Age (years)	48.8±10.2	42.3±11	0.04
Female	9 (34.6%)	6 (23%)	0.73
Male	17 (65.4%)	18 (77%)	0.73
Smokers	10 (38.4%)	5 (21.7%)	0.33
Sedentary lifestyle	15 (57.7%)	12 (52.1%)	0.92
Unhealthy diet	12 (46.1%)	8 (34.7%)	0.6
High blood pressure	24 (92%)	4 (17.3%)	<0.001
FPG >100 mg/dL or diabetes	14 (53.8%)	4 (17.3%)	0.01
BMI (kg/m ²)	16 (61.5%)	11 (42.7%)	0.49
WC (cm)	25 (96.1%)	16 (69.5%)	0.03
WHR	0.91±0.03	0.78±0.03	0.2
Total cholesterol (mg/dL)	235.1±43	203.2±78.6	0.01
HDL-cholesterol (mg/dL)	45.6±8.5	59.4±17.9	0.002
LDL-cholesterol (mg/dL)	153.5±37.6	129.1±36.6	0.02
TG (mg/dL)	22.8±88.8	146.7±78.1	0.002
Insulin (μUI/ml)	14.4±8	11.6±5.4	0.19
HOMA-IR	3.6±1.9	2.65±1.28	0.05
HOMA-IR >2	21 (80.8%)	9 (52.9%)	0.01
SFT (mm)	28.4±11.1	29.8±11.2	0.66
VFT (mm)	91.75±17.4	82.7±24.1	0.05
AST (U/L)	41 (31; 60.7)	34 (29.2; 32.1)	0.07
ALT (U/L)	59 (30.2; 88.5)	54.5 (46.7; 75.2)	0.8
GGT (U/L)	68.5 (42; 112)	48.5 (35.5; 66.5)	0.1

FPG-fasting plasma glucose, BMI-body mass index, WC-waist circumference, WHR-waist/hip ratio, TG-triglycerides, HOMA-IR-Homeostatic model assessment insulin resistance index, SFT-subcutaneous fat thickness, VFT-visceral fat thickness, AST-aspartate aminotransferase, ALT-alanine aminotransferase, GGT-gamma glutamyl transpeptidase, as mean±standard deviation (SD), median and range of 25-75% in parentheses

The prevalence of MS in patients with NASH was 53.1% (Table 1). Regarding the number of MS components, most patients with NASH had three components (34.69%), followed by those with 4 components (28.57%). None of NASH patients had all 5 MS components (Figure 1).

The most common feature of MS was abdominal obesity followed by hypertension and decreased HDL-cholesterol. The prevalence of MS components is shown in Figure 2.

Insulin resistance and correlations with other variables
Patients with NASH had significantly higher values of HOMA-IR compared to control group (Table 2). The prevalence of IR assessed by HOMA-IR ≥ 2 was 69.7% in patients with NASH vs. 43.3% in the control group ($p=0.04$). Prevalence of IR in patients with NASH and MS was significantly higher than in patients with NASH without MS (80.8% vs. 52.9%, $p=0.01$). In univariate analysis, HOMA-IR was positively correlated with WC ($r=0.321$; $p=0.03$), VFT ($r=0.400$; $p<0.001$), BMI ($r=0.230$; $p=0.04$), AST ($r=0.444$; $p<0.001$), ALT ($r=0.320$; $p=0.006$) and GGT ($r=0.343$; $p=0.02$).

HOMA-IR values did not correlate with age, TG, total cholesterol, HDL-cholesterol and LDL-cholesterol levels.

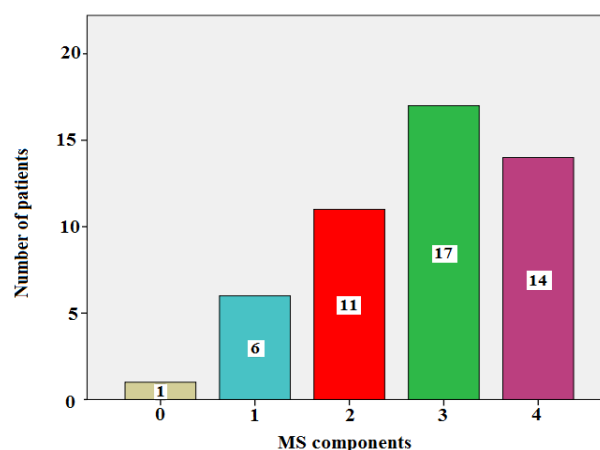


Figure 1. Distribution of MS components in patients with NASH. There are no percentage differences between patients with HOMA-IR ≥ 2 compared to those with HOMA-IR < 2 , in terms of smoking, sedentary lifestyle, unbalanced diet, presence of hypertension, diabetes, hypertriglyceridemia, low HDL-cholesterol (χ^2 test; $p>0.05$).

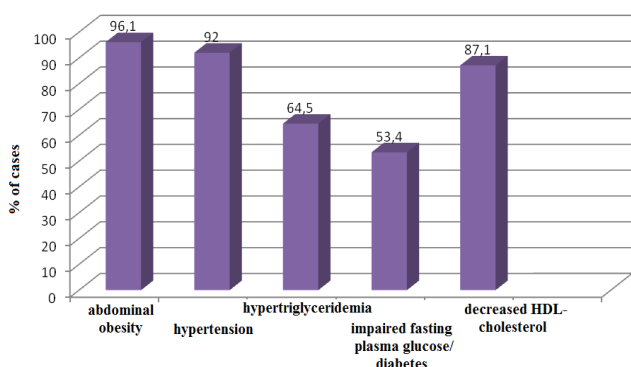


Figure 2. The prevalence of MS components

For HOMA-IR ≥ 2 , multiple linear regression analysis showed that VFT ($p=0.001$) was the only factor independently associated with IR at this threshold.

Abdominal obesity and correlations with other factors
Compared to the control group, patients with NASH had an abdominal distribution of obesity evidenced by the significant increase of WC and VFT and a WHR >1 .

High WC was significantly associated with increased BMI ($r=0.751$; $p<0.001$), VFT ($r=0.442$, $p<0.001$), insulin levels ($r=0.405$; $p=0.007$) and HOMA-IR ($r=0.321$; $p=0.03$).

Correlations between VFT and other clinical and biochemical parameters are shown in Table 3. In the univariate analysis, VFT was positively correlated with BMI, WC, total cholesterol, LDL-cholesterol, TG, IR, AST, ALT, GGT, and negatively with HDL-cholesterol.

Table 3. Spearman’s correlations between VFT and some clinical and biochemical parameters

Variable	VFT	
	r	p
Age (years)	0.163	0.1
BMI (kg/m ²)	0.707	<0.001
WC (cm)	0.674	<0.001
WHR	0.704	<0.001
Total cholesterol (mg/dL)	0.329	0.003
HDL-cholesterol (mg/dL)	-0.401	<0.001
LDL-cholesterol (mg/dL)	0.283	0.01
TG (mg/dL)	0.519	<0.001
FPG (mg/dL)	0.175	0.1
Insulin (μ UI/mL)	0.392	0.001
HOMA-IR	0.4	<0.001
AST (U/L)	0.63	<0.001
ALT (U/L)	0.546	<0.001
GGT (U/L)	0.637	<0.001

BMI-body mass index, WC-waist circumference, WHR-waist/hip ratio, TG-triglycerides, FPG-fasting plasma glucose, HOMA-IR-Homeostatic model assessment insulin resistance index, AST-aspartate aminotransferase, ALT-alanine aminotransferase, GGT-gamma glutamyl transpeptidase

There were no differences in VFT between men (74.8 ± 25.2) and women (70 ± 29.4) ($p=0.4$). Patients with hypertension had higher VFT (92.8 ± 19.6) than those without hypertension (64.2 ± 24.5) ($p<0.001$). Patients with diabetes had higher VFT (106.8 ± 11.4) than those without diabetes (71 ± 25.7) ($p<0.001$). Patients with HOMA-IR ≥ 2 had higher VFT (80.3 ± 26.8) than those with HOMA-IR <2 (60.5 ± 20.6) ($p=0.001$). In multivariate analysis, BMI was the only factor independently associated with VFT ($p=0.03$).

As seen in Figure 2, the area under the curve (AUC), representing VFT as a predictor for NASH in women, was 0.960 (95% CI 0.886-1, $p<0.001$, Figure 3a) and the cut-off value >54.3 mm had a sensitivity of 93.3% (95% CI 55.5-99.7) and a specificity of 90% (95% CI 55.5-99.7). The AUC representing VFT as a predictor for NASH in men was 0.900 (95% CI 0.822-0.978, $p<0.001$, Figure 3b) and the cut-off value of >70 mm had a sensitivity of 70.5% (95% CI 52.5-84.9) and a 100% specificity (95% CI 83.2-100).

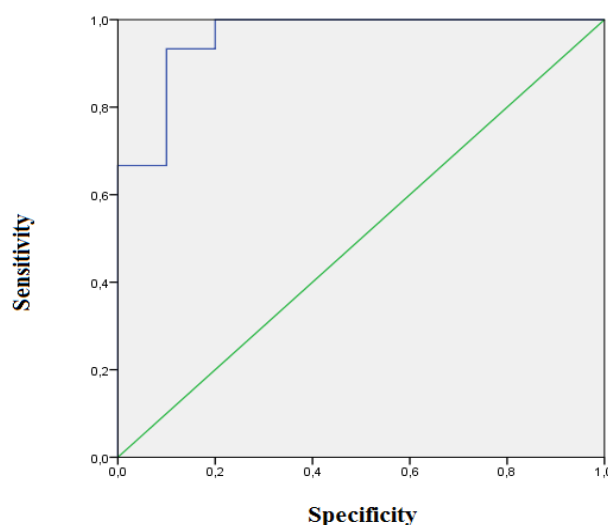


Figure 3. a) ROC curve for VFT in women

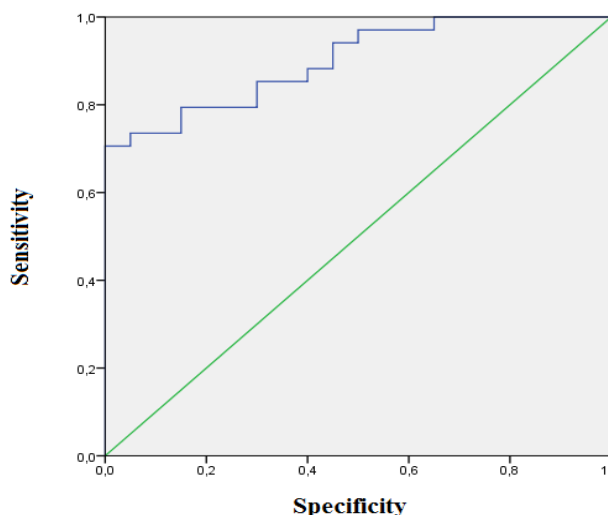


Figure 3. b) ROC curve for VFT in men

Discussion and conclusions

MS includes several clinical and biochemical traits (abdominal obesity, hypertension, impaired FPG or type 2 diabetes, low HDL-cholesterol, hypertriglyceridemia) and it is associated with

significant cardiovascular morbidity and mortality. The prevalence of MS in the general population is approximately 22% with slight differences based on race, sex and age (Ford et al 2002). NAFLD is considered the hepatic component of MS; they both share IR as a pathogenic factor. Over 90% of patients with NAFLD have at least one of the MS features, while 55–62.5% of patients meet all three criteria (Aygun et al 2008, Marchesini et al 2003).

In the present study, we used the last MS accepted definition of the 2009 Consensus (Alberti et al 2009); the prevalence of MS in patients with NASH was 53.1% and of all MS components, abdominal obesity had the highest prevalence, followed by hypertension and decreased HDL-cholesterol. 97.06% of NASH patients had at least one MS component and 63.26% had at least three. However none of the NASH patients had all 5 components. Our data is similar to the literature concerning the prevalence of MS and its components (Marchesini et al 2003). Thus the prevalence of MS is 2–3 times higher in patients with NAFLD compared to the general population but it depends on the diagnostic criteria.

IR and abdominal obesity, both features of MS, are closely associated with the pathogenesis of NASH (Kotronen et al 2008). According to the two-hit theory, first hit is the presence of IR, which triggers the hepatic steatosis and the second hit is represented by a series of liver assaults such as oxidative stress, lipid peroxidation, mitochondrial dysfunction and release of proinflammatory cytokines that are responsible for necroinflammation and fibrosis. IR increases the influx of free fatty acids (FFA) to the liver and de novo lipogenesis, which causes lipid accumulation in hepatocytes (Day et al 1998, Petta et al 2009). In the present study, FPG, insulin levels and HOMA-IR were significantly higher in patients with NASH compared to the control group. The IR prevalence (measured by HOMA-IR ≥ 2) was 69.7% in patients with NASH and increased to 80.8% in patients with associated MS. Higher HOMA-IR was associated with increased abdominal obesity (evaluated by measuring the WC and the VFT). Abdominal obesity assessed by measuring VFT, correlated with IR and increased AST and ALT levels (which are surrogate markers of liver necroinflammation). IR was the only factor independently associated with MS, while VFT was the only factor independently associated with IR. Literature data show that the size of visceral fat is associated with the severity of inflammation and fibrosis in NASH patients (Kuk et al 2006). The results of this study underline once more the close pathogenic relationship that exists between abdominal obesity, IR, MS and NASH.

It is known that abdominal obesity, present in most patients with NASH, plays a central role in the progression of IR, liver inflammation and ATS (Wellen et al 2005). Obesity induced-IR is associated with increased lipolysis in adipose tissue and resulting FFA trigger the activation of liver macrophages, followed by the release of pro-inflammatory cytokines (Maher et al 2008). Growth and inflammation of VAT causes an imbalance in the secretion of various adipokines and cytokines that contribute to the onset and progression of NAFLD, as well as IR and ATS (Matsuzawa et al 2004).

In obese or overweight patients, low-grade inflammation of VAT is one of the leading mechanisms responsible for IR. In lean persons, who do not show a VAT increase, IR occurs due to inhibition of the insulin signaling cascade as a result of lipid accumulation in the skeletal muscle. IR at this level is associated

with hyperinsulinemia that promotes hepatic IR and steatosis by increasing lipogenesis through the SREBP-1c pathway and by inhibiting β -oxidation of fatty acids (Savage et al 2007, Petersen et al 2007).

In this study, visceral obesity was positively correlated with increased TG, total cholesterol and LDL-cholesterol and negatively correlated with decreased HDL-cholesterol. It is well known that abdominal obesity and IR are accompanied by typical atherogenic dyslipidemia, that promotes TG and LDL-cholesterol increase and HDL-cholesterol decrease and it is associated with a 3–7 fold increase of cardiovascular risk compared to non-dyslipidemic subjects (Chapman et al 2011).

IR is also accompanied by an excess of FFA (derived from increased lipolysis and de novo lipogenesis) and Apo B (derived from decreasing degradation), which stimulates the synthesis of very low density lipoproteins (VLDL)-TG (Olofsson et al 2005, Adiels et al 2008).

Limitations of this study derive from the fact that it was a cross-sectional study, the number of patients was relatively small and the time between the liver biopsy and clinical evaluation was relatively long, which did not allow us to make correlations between histological parameters and other variables.

In conclusion, patients with NASH showed a marked increase in MS prevalence compared to the general population. The most common MS components were abdominal obesity, hypertension and low HDL-cholesterol. IR measured by HOMA-IR was positively correlated with abdominal obesity and transaminase levels. Abdominal obesity plays a central role in the development of IR, MS and liver inflammation. VFT showed significant correlations with many cardiovascular risk factors (WC, BMI, WHR, hypertension, diabetes, dyslipidemia and IR). Ultrasound evaluation of VFT proves to be a very good independent predictor of NASH and a simple and non-invasive method for assessing abdominal obesity.

Acknowledgements

The authors would like to express their gratitude towards Professor Maria Dronca, for her valuable advice and supervision of the research.

References

- Adiels M, Taskinen MR, Borén J. Fatty liver, insulin resistance, and dyslipidemia. *Curr Diab Rep* 2008;8:60–64.
- Alberti KM, Ekel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation task force on epidemiology and prevention; National Heart, Lung and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009;120(16):1640–45.
- Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;346:1221–31.
- Aygun C, Kocaman O, Sahin T, Uraz S, Eminler AT, Celebi A, et al. Evaluation of metabolic syndrome frequency and carotid artery intima-media thickness as risk factors for atherosclerosis in patients with nonalcoholic fatty liver disease. *Dig Dis Sci* 2008;53:1352–1357.
- Brunt EM. Nonalcoholic steatohepatitis: pathologic features and differential diagnosis. *Semin Diagn Pathol* 2005;22:330–38.

- Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Boren J, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J* 2011;(32):1345–61.
- Day CP, James OF. Steatohepatitis: a tale of two “hits”? *Gastroenterology* 1998;114(4): 842–45.
- Global atlas on cardiovascular disease prevention and control. In: Mendis S, Puska P, Norrving B, editors. Geneva: World Health Organization (in collaboration with the World Heart Federation and World Stroke Organization); 2011 [http://www.world-heart-federation.org/cardiovascular]
- Finelli C, Sommella L, Gioia S, La Sala N, Tarantino G. Should visceral fat be reduced to increase longevity? *Ageing Res Rev* 2013;12:996-04.
- Freedland ES. Role of a critical visceral adipose tissue threshold (CVATT) in metabolic syndrome: implications for controlling dietary carbohydrates: A review. *Nutrition and Metabolism*, 2004;1(12):1-24.
- Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002;287:356-59.
- Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41(6):1313-21.
- Kotronen A, Yki-Järvinen H. Fatty liver: a novel component of the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2008;28:27-38.
- Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat is an independent predictor of all-cause mortality in men. *Obesity* 2006;(14):336–42.
- Maher JJ, Leon P, Ryan JC. Beyond insulin resistance: innate immunity in nonalcoholic steatohepatitis. *Hepatology* 2008;4:670-88.
- Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis and the metabolic syndrome. *Hepatology* 2003;37:917–23.
- Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2004;24:29–33.
- Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 1985;28:412-19.
- Mottillo S, Filion KB, Genest J, Joseph L, Poirier P, et al. The metabolic syndrome and cardiovascular risk: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010;56:1113–32.
- Olofsson SO, Boren J. Apolipoprotein B is a clinically important apolipoprotein which assembles atherogenic lipoproteins and promotes the development of atherosclerosis. *J Intern Med* 2005; (258):395-410.
- Petta S, Muratore C, Craxi A. Non alcoholic fatty liver disease pathogenesis: The present and the future. 2009;41:615-25.
- Petersen KF, Dufour S, Savage DB, et al. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc Natl Acad Sci USA*. 2007;(104):12587-94.
- Radu C, Grigorescu M, Crisan D, Lupsor M, Constantin D, Dina L. Prevalence and associated risk factors of nonalcoholic fatty liver disease in hospitalized patients. *J Gastrointest Liver Dis* 2008;17(3):255-60.
- Ramilli S, Pretolani S, Muscari A, et al. Carotid lesions in outpatients with nonalcoholic fatty liver disease. *World J Gastroenterol* 2009;15(38):4770-74.
- Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;53:372–84.
- Romero-Gómez M. Insulin resistance and hepatitis C. *World J Gastroenterology* 2006;12:7075-80.
- Sanyal AJ. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002;123:1705-25.
- Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol Rev* 2007;87:507-20.
- Wellen KE, Hotamisligil GS. Inflammation, stress and diabetes. *J Clin Invest* 2005;(115):1111-19.
- WHO. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. WHO Technical Report Series 894. Geneva 2011: World Health Organization (accessed in May 2013). Available at [http://whqlibdoc.who.int/trs/WHO TRS_894.pdf](http://whqlibdoc.who.int/trs/WHO_TRS_894.pdf).

Authors

- Nicoleta V. Leach, 5th Department of Internal Medicine, “Iuliu Hatieganu” University of Medicine and Pharmacy, 16-18-20 Republicii Street, 400015, Cluj-Napoca, Romania, e-mail: nicoleta.leach@umfcluj.com
- Eleonora Dronca, Department of Medical Genetics, “Iuliu Hatieganu” University of Medicine and Pharmacy, 6 Pasteur Street, 400349, Cluj-Napoca, Romania, e-mail: eleonora.dronca@umfcluj.com
- Corina Radu, Regional Institute of Gastroenterology and Hepatology, “Iuliu Hatieganu” University of Medicine and Pharmacy, 19-21 Croitorilor Street, 400162, Cluj-Napoca, Romania, e-mail: corina.radu@umfcluj.com
- Dana Crisan, Regional Institute of Gastroenterology and Hepatology, “Iuliu Hatieganu” University of Medicine and Pharmacy, 19-21 Croitorilor Street, 400162, Cluj-Napoca, Romania, e-mail: dana.crisan@umfcluj.com
- Dorel P. Sâmpolean, 5th Department of Internal Medicine, “Iuliu Hatieganu” University of Medicine and Pharmacy, 16-18-20 Republicii Street, 400015, Cluj-Napoca, Romania, e-mail: dorel.simpelean@umfcluj.ro
- Monica Lupsor-Platon, Medical Imaging Department, “Iuliu Hatieganu” University of Medicine and Pharmacy, 19-21 Croitorilor Street, 400162, Cluj-Napoca, Cluj, Romania, e-mail: monica.lupsor@umfcluj.com
- Mircea D. Grigorescu, Regional Institute of Gastroenterology and Hepatology, “Iuliu Hatieganu” University of Medicine and Pharmacy 19-21 Croitorilor Street, 400162, Cluj-Napoca, Romania, e-mail: m.grigorescu@umfcluj.com

Citation Leach NV, Dronca E, Radu C, Crisan D, Lupsor-Platon M, Sampelean DS, Grigorescu M. Evaluation of metabolic syndrome in patients with non-alcoholic steatohepatitis. HVM Bioflux 2014;6(4):174-181.

Editor Ștefan C. Vesa

Received 1 October 2014

Accepted 24 October 2014

Published Online 24 October 2014

Funding None reported

**Conflicts/
Competing
Interests** None reported