| Fişa suspiciunii de plagiat / Sheet of plagiarism's suspicion | Indexat la: 100/07 |
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| Opera suspicionată (OS)<br>Suspicious work | Opera autentică (OA)<br>Authentic work |
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| OS | PANTEA, I; LEAŞU, T. Non-alcoholic steatohepatitis – a possibile definition criterion for metabolic syndrome. *Bulletin of the Transilvania University of Braşov*, Series VI: Medical Sciences, 2009, Vol. 2, No.51, p.81-86. |
| OA | MARCHESINI, G.; BUGIANESI, E.; FORLANI, G.; CERRELLI, F.; LENZI, M.; MANINI, R.; NATALE, S.; VANNI, E.; VILLANOVA, N.; MELCHIONDA, N. and RIZZETTO, M. Nonalcoholic Fatty Liver, Steatohepatitis, and the Metabolic Syndrome. *Hepatology*, April 2003, Vol. 37, No. 4, p.917-923. |

| Incidența minimă a suspiciunii / Minimum incidence of suspicion | |
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| p.81:01s-p.81:09s | p.917:01s-p.917:06s |
| p.81:09d-p.81:18s | p.917:01d – p.917:07d |
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| p.82:33d-p.83:06s | p.919:40s-p.920:19s |
| p.83:Fig.1 | p.920:Fig.1 |
| p.83:Fig.2 | p.920:Fig.2 |
| p.84:Fig.1 | p.920:Fig.3 |



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## *Retraction of articles:*

1. **PANTEA, I.; LEASU, T. NON-ALCOHOLIC STEATOHEPATITIS–A POSSIBILE DEFINITION CRITERION FOR METABOLIC SYNDROME.** ***Bulletin of the Transilvania University of Braşov, Series VI: Medical Sciences*****, 2009, 2: 51, 81:86**

**Autorii au solicitat retragerea articolelor publicate in B*ulletin of the Transilvania University of Brasov, Series VI: Medical Sciences* datorită faptului că articolele sunt sub suspiciunea de plagiat.**

**Comitetul ştiinţific a decis retragerea acestor articole la cererea autorilor, decizie validata şi în Consiliul Facultăţii de Medicină din 25.06.2013.**

**Braşov**

**25.06.2013**

# Nonalcoholic Fatty Liver, Steatohepatitis, and the Metabolic Syndrome

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**Nonalcoholic fatty liver disease (NAFLD) has been associated with the insulin-resistance syndrome, at present defined as the metabolic syndrome, whose limits were recently set. We assessed the prevalence of the metabolic syndrome in 304 consecutive NAFLD patients without overt diabetes, on the basis of 3 or more criteria out of 5 defined by the U.S. National Institutes of Health (waist circumference, glucose, high-density lipoprotein [HDL]-cholesterol, triglycerides, and arterial pressure). The prevalence of the metabolic syndrome increased with increasing body mass index, from 18% in normal-weight subjects to 67% in obesity. Insulin resistance (Homeostasis Model Assessment method) was significantly associated with the metabolic syndrome (odds ratio [OR], 2.5; 95% CI, 1.5-4.2; $P < .001$). Liver biopsy was available in 163 cases (54%). A total of 120 patients (73.6%) were classified as having nonalcoholic steatohepatitis (NASH); 88% of them had a metabolic syndrome (vs. 53% of patients with pure fatty liver; $P < .0001$). Logistic regression analysis confirmed that the presence of metabolic syndrome carried a high risk of NASH among NAFLD subjects (OR, 3.2; 95% CI, 1.2-8.9; $P = .026$) after correction for sex, age, and body mass. In particular, the syndrome was associated with a high risk of severe fibrosis (OR, 3.5; 95% CI, 1.1-11.2; $P = .032$). In conclusion, the presence of multiple metabolic disorders is associated with a potentially progressive, severe liver disease. The increasing prevalence of obesity, coupled with diabetes, dyslipidemia, hypertension, and ultimately the metabolic syndrome puts a very large population at risk of forthcoming liver failure in the next decades.** (HEPATOLOGY 2003;37:917-923.)

There is a growing concern for nonalcoholic fatty liver disease (NAFLD) in clinical hepatology. A recent survey indicates that NAFLD may account for approximately 80% of cases with elevated liver enzyme levels in the American population, and that 1 in 4 or 5 American adults actually has NAFLD.[1,2] Similar data have been obtained in the Japanese[3] and the Italian[4,5] populations. Although in most cases fatty liver does not progress to more severe liver diseases, approximately 20% to 30% of patients have histologic signs of fibrosis and necroinflammation, indicating the presence of nonalcoholic steatohepatitis (NASH).[6-9] These patients are at higher risk of developing cirrhosis,[10] terminal liver failure,[10] and hepatocellular carcinoma.[11]

NAFLD is mainly associated with obesity,[12,13] diabetes,[6,8,14] hyperdyslipemia,[6,7,15,16] and insulin resistance,[16-19] which are the main features of the recently characterized metabolic syndrome.[20] The borders of the syndrome, previously known as the insulin-resistance syndrome,[21,22] have long been unsettled. Only recently, the Third Report of the National Cholesterol Education Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATPIII])[23] provided a working definition of the metabolic syndrome, based on a combination of 5 categorical and discrete risk factors (central obesity, hypertension, hypertriglyceridemia, low levels of high-density lipoprotein [HDL]-cholesterol, and hyperglycemia), derived from the guidelines of the International Societies or

*Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ATPIII, Adult Treatment Panel III; HDL, high-density lipoprotein; ALT, alanine aminotransferase; BMI, body mass index; OR, odds ratio; 95% CI, 95% confidence interval; AST, aspartate aminotransferase.*

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**Table 1. Anthropometric, Clinical, and Laboratory Data of Patients With NAFLD**

| | All Cases (n = 304) | Men (n = 252) | Women (n = 52) | P* |
|---|---|---|---|---|
| Age (years) | 42.1 ± 12.1 | 39.5 ± 11.0 | 54.7 ± 9.0 | <.001 |
| BMI (kg/m$^2$) | 28.0 ± 4.0 | 27.7 ± 3.6 | 29.4 ± 5.6 | .006 |
| Normal weight | 68 (22%) | 57 (22%) | 11 (21%) | .034 |
| Overweight | 161 (53%) | 140 (56%) | 21 (40%) | |
| Obese | 75 (25%) | 55 (22%) | 20 (39%) | |
| Waist circumference (cm) | 97.6 ± 10.3 | 98.9 ± 9.2 | 91.1 ± 12.4 | <.001 |
| Waist >102 cm (M), >88 (F) | 134 (44%) | 100 (40%) | 34 (63%) | <.001 |
| Waist-to-hip ratio | 0.92 ± 0.06 | 0.93 ± 0.05 | 0.86 ± 0.07 | <.001 |
| Systolic pressure (mm Hg) | 130 ± 14 | 129 ± 13 | 136 ± 15 | <.001 |
| Diastolic pressure (mm Hg) | 82 ± 10 | 82 ± 10 | 87 ± 10 | <.001 |
| Treated for hypertension | 42 (14%) | 31 (12%) | 11 (21%) | .120 |
| Total cholesterol (mg/dL) | 209 ± 46 | 205 ± 45 | 225 ± 49 | .004 |
| HDL-cholesterol (mg/dL) | 46 ± 12 | 46 ± 12 | 50 ± 11 | <.001 |
| Triglycerides (mg/dL) | 172 ± 111 | 171 ± 112 | 175 ± 112 | .817 |
| Treated for hypertriglyceridemia | 10 (3%) | 7 (3%) | 3 (6%) | .383 |
| Fasting glucose (mg/dL) | 98 ± 22 | 96 ± 17 | 105 ± 27 | <.001 |
| Normal fasting glucose | 261 (86%) | 224 (89%) | 37 (71%) | <.002 |
| Impaired fasting glucose | 23 (8%) | 13 (5%) | 10 (19%) | |
| Diabetes | 20 (7%) | 15 (6%) | 5 (9%) | |
| 120-m glucose (mg/dL)† | 121 ± 40 | 120 ± 40 | 126 ± 38 | .452 |
| Impaired glucose tolerance | 50/202 (25%) | 38/178 (21%) | 12/34 (35%) | .011 |
| Fasting insulin (μU/mL) | 15.8 ± 8.4 | 15.8 ± 8.3 | 15.9 ± 9.1 | .837 |
| HOMA-R (%) | 3.90 ± 2.64 | 3.38 ± 2.55 | 4.18 ± 3.01 | .367 |
| HOMA-R >3.0 | 186 (61%) | 151 (60%) | 34 (65%) | .462 |
| Urate (mg/dL) | 6.0 ± 1.2 | 6.1 ± 1.2 | 5.3 ± 1.2 | .014 |
| AST (U/L) | 41.6 ± 25.2 | 38.5 ± 18.7 | 59.1 ± 44.3 | <.001 |
| ALT (U/L) | 82.1 ± 45.2 | 79.3 ± 40.7 | 95.5 ± 61.2 | .018 |
| AST/ALT | 0.54 ± 0.18 | 0.52 ± 0.18 | 0.62 ± 0.19 | .001 |

NOTE. Data are mean ± SD or number of cases and prevalence.
*Unpaired *t* test, $\chi^2$ or Fisher's exact test.
†Number of cases: men, 178; women, 34.

the statements of World Health Organization.[20,23-25] They can easily be measured in clinical practice, and are suitable for epidemiologic purposes.

The metabolic syndrome is highly prevalent in the general population (approximately 22%), with differences in relation to race, gender, and age.[26] It carries an increased cardiovascular morbidity and mortality,[27] which makes an early and correct assessment mandatory. The prevalence of the metabolic syndrome is very high in type 2 diabetes patients, in whom it influences the risk of chronic complications.[28]

The aim of the present report is 2-fold: (1) to measure the prevalence of the metabolic syndrome, based on predefined ATPIII criteria, in a large series of NAFLD patients without overt diabetes; and (2) to verify whether the presence of multiple metabolic disorders, as is the case in the metabolic syndrome, carries a higher risk of the potentially progressive NASH.

## Materials and Methods

***Patients.*** In the period from January 1999 to June 2002, 304 NAFLD patients without a previous diagnosis of diabetes mellitus were consecutively observed. Patients had either been referred to a liver unit for a comprehensive assessment of a previously detected liver disease or were found to have elevated aminotransferase levels during a screening of patients attending a unit for metabolic diseases for a weight-reducing program. All cases had chronic hypertransaminasemia (alanine aminotransferases [ALT] >1.5 × upper normal values for 3 months or more), negative hepatitis B and C viral markers, absence of autoantibodies indicative of autoimmune hepatitis or celiac disease, and negative or negligible alcohol consumption (<140 g/wk). Alcohol abuse was also excluded by interviewing patients' relatives.

All cases underwent a complete clinical, anthropometric, and laboratory investigation (Table 1) and an ultrasound scan of the liver. Brightness and posterior attenuation were considered indices of the extent of fatty infiltration and fibrosis.[29] According to the severity of the clinical picture and the release of informed consent, 163 cases (53.6%) had a percutaneous liver biopsy performed on day-hospital admission. Laboratory investigations included an oral glucose tolerance test in 205 cases.

**Table 2. Positive Criteria for the Metabolic Syndrome in the Whole Population (number positive and prevalence)**

| | Men (n = 252) | Women (n = 52) | P* |
|---|---|---|---|
| Waist circumference >102 cm (men), >88 cm (women) | 100 (40%; 34-46) | 34 (65%; 51-76) | <.001 |
| Fasting glucose ≥110 mg/dL | 28 (11%; 8-15) | 15 (29%; 17-41) | .002 |
| HDL-cholesterol <40 mg/dL (men) or <50 (women) | 78 (31%; 25-37) | 29 (56%; 41-67) | .001 |
| Triglycerides ≥150 mg/dL or treated with fibrates | 116 (46%; 40-52) | 28 (54%; 40-66) | .360 |
| Arterial pressure ≥130/85 mm Hg or pharmacologically treated | 140 (56%; 49-61) | 40 (77%; 63-85) | .005 |

NOTE. Data are the number positive and prevalence, 95% CI.
*Fisher's exact test.

The 5 components of the metabolic syndrome were available in all patients, and subjects having 3 or more of the following criteria were labeled as metabolic syndromes[23]: (1) fasting glucose ≥110 mg/dL; (2) central obesity (waist circumference >102 cm [men] and >88 cm [women]); (3) arterial pressure ≥130/85 mm Hg or pharmacologically treated; (4) triglyceride levels >150 mg/dL or current use of fibrates; and (5) HDL-cholesterol <40 mg/dL (men) and <50 mg/dL (women).

The study was part of different diagnostic and intervention studies approved by the ethical committee. All subjects gave informed consent for participation.

***Methods.*** Body weight was measured in light clothing and without shoes to the nearest half kilogram. Height was measured to the nearest half centimeter. Body mass index (BMI) was calculated as weight (kg) divided by height$^2$ (m). Waist circumference (at the nearest half centimeter) was measured at the midpoint between the lower border of the rib cage and the iliac crest, whereas hip circumference was similarly obtained at the widest point between hip and buttock.

Three blood pressure readings were obtained at 1-minute intervals, and the second and third systolic and diastolic pressure readings were averaged and used in the analyses.

Plasma glucose, both in the fasting state and in response to a standard glucose load, was measured in duplicate with an automated analyzer. The coefficient of variation for any single determination was ±1.5%. Insulin was measured by an immunoenzymometric assay (AIA-PACK IRI, AIA-1200 system; Tosoh Co., Tokyo, Japan) with intra- and interassay coefficients of variation for the quality control <7%. Cholesterol, HDL-cholesterol, and triglycerides were measured by enzymatic, colorimetric methods, using CHOL, HDL-C plus (2nd generation) and TG assays (Roche Diagnostics Co., Indianapolis, IN). Insulin resistance was calculated by means of the homeostasis model assessment (HOMA-R).[30]

Liver biopsy was scored according to Brunt et al.,[31] with minor modifications. The amount of fat, always present in our biopsies, was graded 1 to 3, according to the percentage of cells with fatty droplets (1, 0%-33%; 2, 34%-66%; 3, 67%-100%). Fibrosis was graded 0 (absent) to 4 (1, perisinusoidal/pericellular fibrosis; 2, periportal fibrosis; 3, bridging fibrosis; 4, cirrhosis). Necroinflammation was graded 0 (absent) to 3 (1, occasional ballooned hepatocytes and no or very mild inflammation; 2, ballooning of hepatocytes and mild-to-moderate portal inflammation; 3, intra-acinar inflammation and portal inflammation). NASH was defined by the presence of fibrosis (grade 1 or more) or necroinflammation (grade 2 or more).

***Statistical Analyses.*** All data were analyzed using StatView 5.0 (SAS Institute Inc., Cary, NC). Unpaired $t$ test (2-tail), $\chi^2$ contingency test, and Fisher's exact test were used whenever appropriate. Nonparametric methods were also used for non-normally distributed values. Logistic regression analysis (univariate analysis) was used to calculate the risk for the metabolic syndrome associated with the presence of the 5 individual components, and the risk for NASH and severe histopathologic lesions in patients with the metabolic syndrome. All analyses were adjusted for age and sex. The odds ratio (OR), the 95% confidence intervals (CI), and $P$ values were calculated. Data in the text and in the tables are reported as mean ± SD.

## Results

There were significant differences in clinical, laboratory, and anthropometric data in relation to gender. Female patients were older, had a higher BMI and higher blood glucose, arterial pressure, and transaminase levels (Table 1).

The prevalence of positive criteria for the metabolic syndrome was exceedingly variable in the whole population, ranging from 11% (hyperglycemia, men) to 77% (hypertension, women) (Table 2). According to increased BMI class (Fig. 1), hyperglycemia nearly doubled from 12% to 21% ($P = .120$), hypertriglyceridemia was as high as 64% ($P = .002$), a low HDL-cholesterol level was present in 30% to 42% of cases and the arterial pressure criteria were fulfilled in 34% to 83% of cases. Finally, waist circumference exceeded the threshold for abdominal obesity in the large majority of obese patients, but also

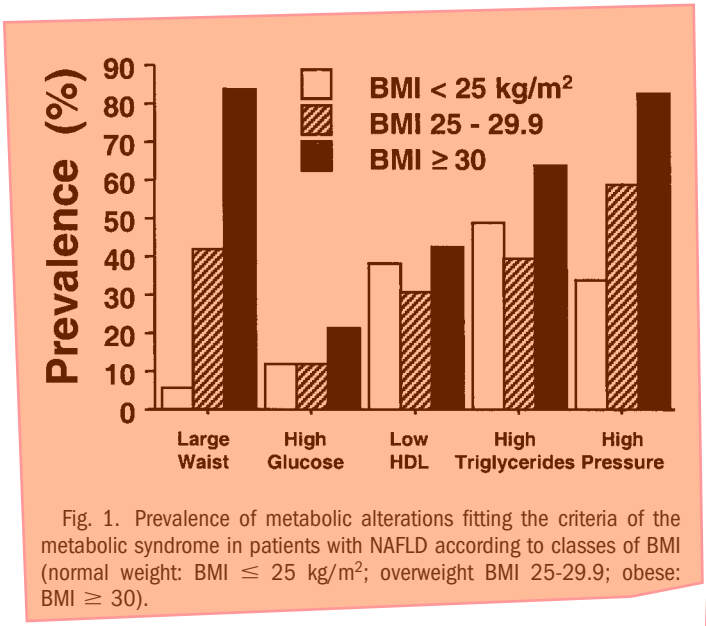


Fig. 1. Prevalence of metabolic alterations fitting the criteria of the metabolic syndrome in patients with NAFLD according to classes of BMI (normal weight: BMI $\leq$ 25 kg/m$^2$; overweight BMI 25-29.9; obese: BMI $\geq$ 30).

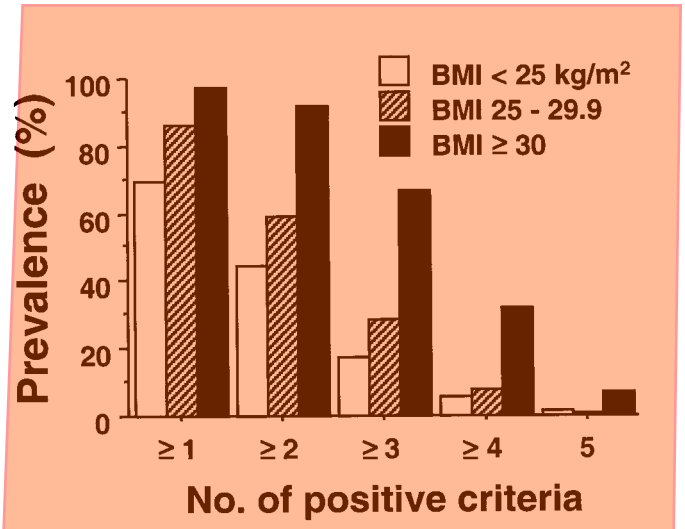


Fig. 2. Prevalence of positive criteria for the metabolic syndrome in patients with NAFLD according to classes of BMI (normal weight: BMI $\leq$ 25 kg/m$^2$; overweight: BMI 25-29.9; obese: BMI $\geq$ 30). Note that the presence of 3 or more positive criteria defines the metabolic syndrome.

in 42% of overweight subjects and 6% of patients with normal BMI.

Only a few patients did not satisfy any criteria of the metabolic syndrome (31% normal weight, 14% overweight, 3% obese; Fig. 2). The cumulative presence of positive criteria was diagnostic for the metabolic syndrome (3 or more criteria) in 36% of total cases (18% of normal weight subjects, 29% overweight, and 67% obese). In 7 cases (2.3%, 1 normal weight, 1 overweight, 5 obese) all 5 criteria were fulfilled. Female sex was more frequently involved (60% vs. 31% in men; $P \leq .0001$, Fisher's exact test). The prevalence of the metabolic syndrome also increased with increasing age (26% in subjects aged less than 40 years to 45% in subjects aged 40 or more; $P = .0005$).

The presence of the metabolic syndrome was significantly associated with female sex (OR, 3.08; 95% CI, 1.57-6.02) and age (OR, 1.54; 95% CI, 1.23-1.93 per 10 years) after adjustment for BMI class. After adjustment for gender and age, a fasting blood glucose $\geq$110 mg/dL was the most predictive criterion for the metabolic syndrome (OR, 18.9; 95% CI, 6.8-52.7), followed by hypertriglyceridemia, hypertension, low HDL-cholesterol, and finally enlarged waist circumference (OR, 4.5; 95% CI, 2.7-7.7).

Insulin resistance (HOMA method) was present in a large proportion of cases. HOMA-R increased on average from 3.48% to 3.69% in normal-weight subjects to 3.88% to 2.34% in overweight and to 4.24% to 1.98% in obese, without differences between groups. In particular, a HOMA-R $\geq$3, assumed as a cutoff point of an insulin-resistant state, was demonstrated in 30 of 68 normal-weight subjects (44%), in 100 of 161 (62%) overweight cases and in 55 of 75 (73%) obese patients ($\chi^2$, $P = .0015$). The presence of insulin resistance, defined as above, was significantly associated with the metabolic syndrome (OR, 2.5; 95% CI, 1.5-4.2; $P < .001$).

Patients who underwent liver biopsy were fairly representative of the whole population. When compared with subjects who did not undergo liver biopsy, they were younger (40 $\pm$ 11 years vs. 44 $\pm$ 13; $P = .004$), with no differences in average glucose and lipid levels, as well as in arterial pressure. Only aminotransferase levels were higher (ALT, 91 $\pm$ 51 vs. 72 $\pm$ 35; $P = .0001$). Among individual criteria for the metabolic syndrome, only the prevalence of hypertriglyceridemia was different and lower in the liver biopsy group (42% vs. 54%; $P = .038$, Fisher's exact test) (Fig. 3), but the overall prevalence of the met-

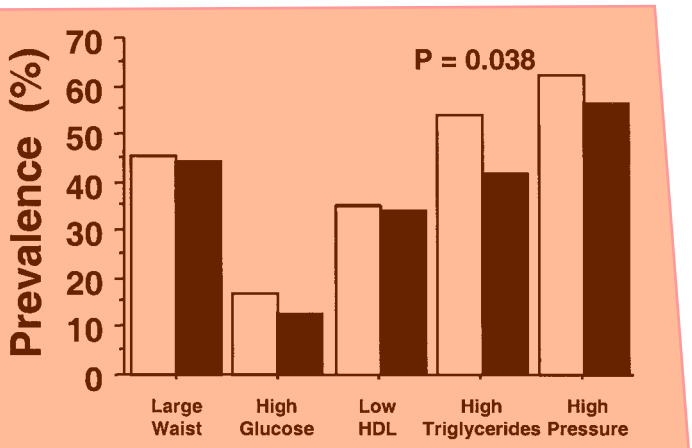


Fig. 3. Prevalence of positive criteria for the metabolic syndrome in NAFLD patients who only had a clinical and laboratory assessment (**open columns,** n = 141) or had a percutaneous liver biopsy (**closed columns,** n = 163). The overall prevalence of the metabolic syndrome was not different.

abolic syndrome was not different (47% in liver biopsy patients vs. 53%; $P = .151$).

On the basis of liver biopsy, 120 cases were classified as NASH (73.6%), 43 as pure fatty liver. Four patients had a liver biopsy indicative of cirrhosis. The prevalence of NASH was not significantly associated with gender (men, 72%; women, 89%; $P = .163$, Fisher's exact test), age (<40 years, 70%; ≥40, 78%; $P = .218$), and increasing BMI class (normal weight, 65%; overweight, 73%; obese, 84%; $P = .184$). However, NASH patients had a more severe metabolic disorder and a larger prevalence of criteria for the metabolic syndrome, compared with subjects with pure fatty liver (Table 3). The metabolic syndrome was present in 88% of NASH patients and only in 67% of fatty liver ($P = .004$, Fisher's exact test). The severity of liver cell necrosis, measured by ALT and aspartate aminotransferase (AST) levels, was different, but it was of no use for diagnostic purposes. Of the 5 criteria for the metabolic syndrome, only hyperglycemia/diabetes was significantly associated with NASH after correction for age, gender, and obesity, but the simultaneous presence of 3 or more criteria, *i.e.*, a defined metabolic syndrome, was associated with a different histopathologic grading, including a higher prevalence of fibrosis and necroinflammatory activity (Table 4). Logistic regression analysis confirmed that the presence of the metabolic syndrome carried a higher risk of NASH (OR, 3.2; 95% CI, 1.2-8.9; $P = .026$), after correction for sex, age, and BMI. In particular, the presence of the metabolic syndrome was not associated with a higher risk of severe steatosis (OR, 0.9; 95% CI, 0.3-3.2; $P = .935$) or severe necroinflammatory activity (OR, 2.0; 95% CI, 0.5-8.3; $P = .334$), but carried a high risk of combined grade 3 or 4 fibrosis (OR, 3.5; 95% CI, 1.1-11.2; $P = .032$).

**Table 3. Clinical and Laboratory Data in Patients With Pure Fatty Liver and NASH (mean ± SD or prevalence and 95% confidence intervals)**

| | Fatty Liver (n = 43) | NASH (n = 120) | P* |
|---|---|---|---|
| Men | 95% (83-98) | 86% (78-91) | .163 |
| Age (y) | 39.1 ± 10.1 | 40.9 ± 12.0 | .318 |
| BMI (kg/m$^2$) | 26.8 ± 2.7 | 28.3 ± 4.1 | .008 |
| Waist circumference (cm) | 96.8 ± 6.5 | 99.8 ± 9.2 | .024 |
| Enlarged waist circumference† | 57% (46-65) | 73% (61-81) | .037 |
| Systolic pressure (mm Hg) | 125 ± 12 | 132 ± 15 | .006 |
| Diastolic pressure (mm Hg) | 79 ± 8 | 82 ± 9 | .009 |
| Hypertension† | 53% (42-53) | 72% (62-80) | .011 |
| Total cholesterol (mg/dL) | 210 ± 42 | 208 ± 46 | .746 |
| HDL-cholesterol (mg/dL) | 48 ± 14 | 45 ± 11 | .107 |
| Low HDL-cholesterol† | 57% (48-66) | 76% (62-84) | .019 |
| Triglycerides (mg/dL) | 140 ± 88 | 169 ± 106 | .069 |
| Hypertriglyceridemia† | 58% (48-63) | 72% (60-81) | .054 |
| Fasting glucose (mg/dL) | 92 ± 10 | 98 ± 25 | .069 |
| Hyperglycemia† | 60% (51-67) | 91% (69-96) | .004 |
| 120-m glucose (mg/dL)‡ | 122 ± 38 | 122 ± 40 | .969 |
| Fasting insulin (μU/mL) | 14.1 ± 8.1 | 16.9 ± 8.9 | .040 |
| HOMA-R (%) | 3.25 ± 2.03 | 4.16 ± 2.61 | .018 |
| AST (U/L) | 37.5 ± 15.3 | 46.9 ± 31.9 | .030 |
| ALT (U/L) | 78.5 ± 35.0 | 96.0 ± 56.6 | .028 |
| AST/ALT | 0.51 ± 0.18 | 0.51 ± 0.17 | .928 |

NOTE. Data are mean ± SD or prevalence and 95% CI.
*Unpaired *t* test or Fisher's exact test.
†Cutoff according to criteria for metabolic syndrome (see text).
‡Number of cases: fatty liver, 37; NASH, 85.

**Table 4. Histopathologic Grading of Liver Biopsies in NAFLD Patients According to the Presence of the Metabolic Syndrome**

| | With Metabolic Syndrome (n = 51) | Without Metabolic Syndrome (n = 112) | P* |
|---|---|---|---|
| Fatty infiltration | | | .306 |
| Mild | 23 (45%, 31-58) | 65 (58%, 48-66) | |
| Moderate | 20 (39%, 26-52) | 33 (29%, 21-38) | |
| Severe | 8 (16%, 7-27) | 14 (12%, 7-19) | |
| Fibrosis | | | .0005 |
| Absent | 6 (12%, 5-22) | 46 (41%, 32-50) | |
| Perisinusoidal/pericellular | 7 (14%, 6-25) | 21 (19%, 12-26) | |
| Periportal | 21 (41%, 28-54) | 28 (25%, 17-33) | |
| Bridging | 14 (27%, 16-40) | 16 (14%, 9-21) | |
| Cirrhosis | 3 (6%, 1-15) | 1 (1%, 0-4) | |
| Necroinflammation | | | .031 |
| Absent | 3 (6%, 2-15) | 18 (16%, 10-23) | |
| Mild | 17 (33%, 21-46) | 45 (40%, 31-49) | |
| Moderate | 29 (57%, 42-69) | 49 (44%, 34-53) | |
| Severe | 2 (4%, 1-12) | 0 (0%, 0-2) | |

NOTE. Data are the number positive and prevalence, 95% CI.
*$\chi^2$ test.

## Discussion

The present report further highlights the association of NASH with features of the metabolic syndrome. Obesity, diabetes, hypertension, and hyperlipidemia have been repeatedly reported in NAFLD, but their simultaneous presence significantly increases the risk of more severe stages of liver disease. This association is maintained also in the present group of patients without overt diabetes, where the very high prevalence of the metabolic syndrome might blur the relationship.

The cutoff points for the metabolic syndrome derive from accepted standards. They indicate the upper limits for "normal" subjects. As far as central obesity is concerned, the limits are also fulfilled by several patients in the overweight range and by very few cases with normal BMI. This policy agrees with the major role of central obesity in the pathogenesis of the metabolic syndrome,[32] but nonetheless fails its scope. In our series, abdominal adiposity was the least predictive factor for the metabolic syndrome. It might result from a selection bias, because obese and morbidly obese patients are not immediately referred to liver units in the presence of mild-to-moderate

hypertransaminasemia, but are primarily managed by dieting or lifestyle changes for weight reduction.

There is no consensus as to the best indicator of abdominal adiposity.[33] The use of the sole waist circumference identifies a larger proportion of women, in contrast with the common belief linking abdominal adiposity to the male sex. Similar data were reported by Ford et al.[26] in their survey of a U.S. population. In selected groups, a lower cutoff point of 94 cm for waist circumference was suggested by ATPIII.[23] Such a limit would increase the prevalence of the metabolic syndrome in our male population from 31% to 38% and the overall prevalence from 36% to 42%.

A correct identification of subjects with the metabolic syndrome is mandatory, because these patients are generally at higher risk of cardiovascular disease,[27,34] particularly in the presence of diabetes.[28] The overall prevalence of the metabolic syndrome is on average 22% in the adult U.S. population[26] and around 10% to 15% in Finland.[34] It increases steadily with increasing age, and doubles from the age range of 40-49 to over 60.[26] There are no epidemiologic data on the Italian population, where the overall prevalence of diabetes and obesity is only moderately lower than that observed in the United States. Considering that the median age of our patients was 40 years, the prevalence of the metabolic syndrome may be approximately doubled in NAFLD compared with the general population.

The presence of the metabolic syndrome was significantly associated with insulin resistance. A central role for insulin resistance in the pathogenesis of NAFLD is supported by physiopathologic considerations, clinical association, and laboratory investigations. In this series, insulin resistance was assessed by the HOMA method,[30] a rather crude index of insulin action. However, HOMA significantly correlates with clamp techniques,[35] the gold standard for the measurement of insulin sensitivity, and can be practically used for large surveys. HOMA values were high in most NAFLD patients, but increased HOMA values were associated with an increased risk of the metabolic syndrome, further supporting a pathogenic role.

The histologic criteria to define NASH remain tentative. Following the evidence of progression of liver disease also in subjects with ballooning degeneration,[9] we classified as NASH all patients with histologic signs of hepatocellular damage and/or fibrosis of any degree. Accordingly, the relative proportion of NAFLD patients classified as NASH was very high (over 70%), and higher than usually reported.[1,2] In random histopathologic studies, the relative proportion of NASH to NAFLD is approximately 1:10.[36,37] This problem has never been systematically evaluated, and selection biases might operate differently in the various series. In the present study, liver biopsy was limited to a subgroup of patients, and it is possible that more severe cases were preferentially selected or gave informed consent to biopsy. Certainly, selection of patients for liver biopsy was not based on coexisting diseases. The prevalence of individual criteria for the metabolic syndrome was not systematically different in patients who received a liver biopsy, who were fairly representative of the whole series. It is also possible that only cases with more severe or more prolonged forms of liver disease were seen at specialist centers, thus increasing the relative prevalence of NASH. This conclusion is supported by significant differences in enzyme levels between pure fatty liver and NASH. However, this difference was only valuable on statistical ground, and amplified by the large number of cases. In obesity, ALT levels did not predict the extent of histologic severity of liver disease[13]; in a previous series of NAFLD, aminotransferase levels did not differ in relation to the severity of steatosis, necroinflammatory infiltration, and fibrosis.[38] Accordingly, aminotransferase levels cannot be confidently used as surrogate markers of disease in intervention studies, which constitutes a severe limit in the assessment of the effects of lifestyle changes, dieting, and drugs on disease progression.

A selection bias might also be the reason for the higher prevalence of the metabolic syndrome in the female sex, whereas the higher prevalence with increasing age may be easily explained by the higher prevalence of obesity and altered glucose regulation with advancing age.[39] Anyway, these factors are no longer present in the risk of NASH, which remains strictly associated with the presence of the metabolic syndrome.

The association of the metabolic syndrome with NASH and with more severe necroinflammatory activity and fibrosis is particularly relevant in epidemiologic terms. The prevalence of obesity is dramatically increasing in the western world, assuming the character of an epidemic.[24] Obesity in turn increases the prevalence of diabetes,[39] dyslipidemia, hypertension, and ultimately of the metabolic syndrome. Our analysis underlines the existence of an exceedingly large population at risk of forthcoming liver failure in the next decades, provided they survive the burden of cardiovascular disease mortality.[34]

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