The Risk of Colorectal Adenomas in Patients with Nonalcoholic Fatty Liver Disease (NAFLD). A Case-Control, Prospective Study

INTRODUCTION
Colorectal carcinoma (CRC) is a serious health issue [1]. The early detection of colorectal adenomas through colonoscopy has led to a lower mortality rate in left colorectal cancers, but not in right colorectal cancers [1,2]. The panchromoendoscopy with specific dyes improves the detection rate of colorectal adenomas, especially the plane ones or those of the right colon. Hyperinsulinemia and insulin resistance are two factors involved in the pathogenesis of colorectal cancer. Numerous studies have proven that obesity and diabetes mellitus are two diseases which are more frequently associated with colorectal adenomas and carcinomas [3,4,5]. The non-alcoholic fatty liver is associated with liver insulin resistance. The association of colorectal adenomas and carcinomas with nonalcoholic fatty liver disease is currently being assessed. The non-alcoholic fatty liver is associated with liver insulin resistance. The most recent data shows that patients with fatty liver have a higher risk of colorectal adenomas and carcinomas, especially on the right colon, than those without liver steatosis [6,7,8].

In this study we aimed at assessing the detection rate of colorectal adenomas and advanced neoplasms evaluated with specific dyes in patients with NAFLD. We included in the study 100 patients with or without NAFLD who underwent panchromoendoscopy. NAFLD with or without nonalcoholic steatohepatitis (NASH) was diagnosed by liver biopsy or by abdominal ultrasounds followed by laboratory tests to eliminate the other causes of steatosis. We detected 12 colorectal advanced neoplasms and 52 adenomas. No difference in the detection rate of adenomas (32% vs. 22%; p=0.260), but there was difference in the prevalence of advanced colorectal neoplasms (20% vs. 4%; p<0.014) between groups. Multivariate regression analysis demonstrated an independent risk of colorectal adenomas with NASH after adjustment, according to demographic and metabolic factors (OR= 9.52; p=0.010). The prevalence of adenomas and carcinomas is higher in patients with NAFLD than non-NAFLD patients. NASH is an independent factor that is associated with the presence of colorectal adenomas.

MATERIALS AND METHODS
The study is a case-control, prospective study. The protocol of the study was approved by the Ethical Committee of the University of Medicine and Pharmacy “Iuliu Hatieganu” from Cluj-Napoca in December 2011.

Patients
Study participants were prospectively recruited from subjects who were planned to undergoing a colonoscopy for different symptoms (not screening, because in Romania screening program for colorectal cancer does not exist) at the 4th Medical Clinic Cluj-Napoca, from January 2012 to April 2013.

All patients aged between 35 and 75 years, which are included in the study, agreed with the study data and signed the informed consent. We have screened 230 consecutive patients.

An abdominal ultrasounds, medical history and laboratory tests were performed before colonoscopy.

Information on medical history, current use of medications, daily alcohol consumption was obtained from all participants using a standard questionnaire. Patients with alcohol intake over 20g/day, infectious hepatitis, acute or chronic liver disease, hereditary liver disease and immunological liver damage, family and personal history of colorectal adenoma or carcinomas, patients with inflammatory bowel disease were excluded from the study.

Were excluded from study 111 patients (43 patients intake over 25 g/day of alcohol, 2 were diagnosed with chronic hepatitis B, 5 with chronic hepatitis C, 2 patients with autoimmune hepatitis, 3 with hemocromatosis, 32 patients had personal history of adenomatous polyps, 8 patients had personal history of colorectal cancer and 16 were previously diagnosed with inflammatory bowel disease).

For the first group (NAFLD group) we enrolled either patients with steatosis on abdominal ultrasounds, negative medical history of liver disease or drug induced-hepatitis, negative

ABSTRACT
Few studies have evaluated the prevalence of colonic adenomas and carcinomas on patients with NAFLD. We included in the study 100 patients with or without NAFLD who underwent panchromoendoscopy. NAFLD with or without nonalcoholic steatohepatitis (NASH) was diagnosed by liver biopsy or by abdominal ultrasounds followed by laboratory tests to eliminate the other causes of steatosis. We detected 12 colorectal advanced neoplasms and 52 adenomas. No difference in the detection rate of adenomas (32% vs. 22%; p=0.260), but there was difference in the prevalence of advanced colorectal neoplasms (20% vs. 4%; p<0.014) between groups. Multivariate regression analysis demonstrated an independent risk of colorectal adenomas with NASH after adjustment, according to demographic and metabolic factors (OR= 9.52; p=0.010). The prevalence of adenomas and carcinomas is higher in patients with NAFLD than non-NAFLD patients. NASH is an independent factor that is associated with the presence of colorectal adenomas.

Keywords
colorectal adenomas, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, panchromoendoscopy

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blood tests for other causes of steatosis or patients with hepatic liver biopsy consisted of NAFLD with or without different staging of NASH.

Liver biopsy was proposed in patients with steatosis on abdominal ultrasounds, negative medical history of liver disease or drug induced-hepatitis, negative blood tests for other causes of steatosis but with abnormal liver biochemistry (abnormal level of aminotransferases). Sixteen patients were agreed with liver biopsy and 2 rejected the procedure. At the end, only 50 patients were included in NAFLD group (34 without liver biopsy and 16 patients with liver biopsy).

The second group (control group) consisted of patients without steatosis on abdominal ultrasounds and who was planned for a colonoscopy for different reasons and who agreed with our methodology of study. For statistically reason we limited this group at 50 consecutive patients, so we excluded the last consecutive 17 patients without steatosis on abdominal ultrasonography.

In all patients (case and control) demographic data (name, age, environmental status) and anthropometric measurements (weight, BMI, waist circumference) were recorded, blood pressure was measured, biochemical analysis were performed, as well as abdominal ultrasounds and panchromoendoscopy.

In patients with colorectal polyps and tumors on colonoscopy, a histological exam was made. The body mass index (BMI) for all patients was calculated in kg/m². Increased waist circumference was defined as waist greater than 94 cm in men and greater than 80 in women. The blood pressure was measured in all patients. Patients with antihypertensive drugs and blood pressure over than ≥140 mmHg and /or ≥90mmHg at consecutive two measurements were diagnosed with hypertension.

Also, the presence of components of metabolic syndrome such as: increased waist circumference, high blood pressure, type 2 diabetes mellitus/glucose intolerance, hypertriglyceridermia, low HDL-cholesterol were recorded in all patients included in the study.

The metabolic syndrome was diagnosed when there 3 criteria from 5 have met: increased waist circumference, high blood pressure, type 2 diabetes mellitus/glucose intolerance, hypertriglyceridermia or low HDL-cholesterol.

Abdominal ultrasound
Hepatic abdominal ultrasound was carried out for all screen patients, by one of five sonographers with more than 5 years experience. The hepatic abdominal ultrasounds were done before colonoscopy, in order to detect the presence of liver steatosis. This is defined by the presence of increased echogenicity of the liver accompanied by deep beam attenuation with impaired diaphragm visualization, contrast liver to kidney, bright vessels walls and impaired gallbladder walls visualization [9].

Laboratory exams
Fasting blood was taken from all category of patients for the following analysis: total blood count cell, transaminases, alkaline phosphatase, gamma glutamyl transferase, total bilirubin, serum electrophoresis, glucose, glucose tolerance test, HDL-cholesterol, LDL-cholesterol, triglycerides, Surface Hepatitis B Antigen, Hepatitis C Virus Antibodies, Antinuclear antibodies, Mitochondrial Antibodies, Liver Kidney Microsomal type 1 Antibodies, Soluble Liver Antigen , blood iron levels, ferritin, transferrin saturation coefficient, ceruloplasmin levels.

Ultrasound-guided liver biopsy
Using an ultrasonograph Aloka 7 (Hitachi Aloka Medical, Ltd.), an ultrasound-guided liver biopsy was made in patients with liver steatosis on abdominal ultrasounds and abnormal liver biochemistry who were included in study and were agree and signed an standard inform consent for liver biopsy (from 18 patients, 2 of patients were not agree with liver biopsy and were excluded). An interventional ultrasonographer with more than 10 years experience made all liver biopsies. A single pass ultrasound-guided liver biopsy was made in each patient, after a local anesthesia with 10 ml of Lidocain sol. 2%. The liver biopsies were performed with a Bard Tru-Cut biopsy needle of 22 Ga in diameter. A tissue fragment larger than 5 mm was considered sufficient for histological exam. If the material was insufficient a second pass was made.

Colonoscopy-panchromoendoscopy
Following bowel preparation with 4 L polyethylene glycol lavage solution, all patients underwent total colonoscopy under sedation. Total chromoendoscopy with 0.4% indigo carmine dye was carried out only by one endoscopist with experience in chromoendoscopy. In the patient study chart the number of polypoid or non-polypoid colonic polyps, the size, location and type of endoscopic resection were recorded.

Histopathology exam
All liver specimens from liver biopsy were sent to histopathology exam. According to American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association Guideline, NAFL was defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes. NASH was defined as the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis [10].

All biopsied or removed lesions from colonoscopy were sent to histopathology exam, to assess polyph or tumor histology. Non-adenomatous polyps were excluded from the study. Adenomatous polyps were evaluated according to histological type, presence of dysplasia, grade of dysplasia and presence of carcinoma. Advanced colorectal neoplasm was defined as cancer or adenomas with villous architecture or high grade dysplasia or adenoma larger than 10mm in diameter. Invasive colorectal cancer was defined by the presence of tumor cells in the submucosa.

Statistical analysis
The statistical analysis was performed with the IBM SPSS program version 19 (trial version). The statistical results are presented as the mean ± standard deviation or percentages. Statistical analyses included one-way analysis of variance (ANOVA), Student’s t-test, Pearson Chi Square (Hi²), Fisher test, robust test of Brown-Forsythe and post-test analysis- Tukey HSD. The relationship of NAFLD with the presence of adenomatous polyps was assessed by multiple logistic regression analysis after adjustment for independent variables, including age, sex, BMI, waist circumference, diabetes and NASH. Each odds ratio (OR) is presented together with its 95% confidence interval (CI), p<0.05 was considered statistically significant.

RESULTS
From 230 patients who were screened, we excluded 113 patients from the study because they had one or more exclusion criteria. Furthermore, we limited at 50, patients in the control group for statistically reasons, so we also excluded the consecutive last 17 patients from control group. Finally, out of 100 patients were included in the study, 50 were with NAFLD and 50 were without NAFLD. Out of 50 patients, 34 patients were diagnosed with NAFLD based positive abdominal ultrasound for steatosis, negative history for alcohol intake, hereditary, chronic or drug-induced hepatitis, and negative serological tests (infectious, alcoholic, immunological, and hereditary). In sixteen patients (steatosis on abdominal ultrasonography and abnormal liver
biochemistry), the NAFLD was proven through liver biopsy (all patients were diagnosed with different grading of NASH).

Median age was 59.14 ±8.5 years in NAFLD group and 58.26 ±13.2 years in controls with no statistically difference. The women were predominant (31/50, 62%) then men (19/50, 38%) in both groups. Baseline characteristics, anthropometric measurements, clinical and biochemical data of the participants in this study can be seen in table nr.1.

Table 1, about baseline characteristics, anthropometric measurements, and biochemical data of the study participants with NAFLD and control patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>NAFLD group N=50</th>
<th>Control group N=50</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.14 ±8.5</td>
<td>58.26 ±13.2</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>F: females; M: men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>30 (60%)</td>
<td>32 (64%)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.17 ±5.4</td>
<td>25.42 ±4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IC</td>
<td>43 (86%)</td>
<td>7 (14%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyper-Trigly</td>
<td>24 (48%)</td>
<td>26 (52%)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Low HDL-Chol</td>
<td>32 (64%)</td>
<td>18 (36%)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>HTA</td>
<td>30 (60%)</td>
<td>20 (40%)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>DM</td>
<td>13 (26%)</td>
<td>37 (74%)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>MS</td>
<td>32 (64%)</td>
<td>16 (32%)</td>
<td>&gt;0.001</td>
</tr>
</tbody>
</table>

We detected 29 adenomas on distal colon in NAFLD group and only 4 adenomas on proximal colon with no differences between groups. However, when the total number of polyps were counted (hyperplastic polyps included) a statistical difference between groups was detected (p=0.024). Patients with NAFLD had statistically more polyps (adenomas and hyperplastic) than controls.

In the NAFLD group, the risk of colorectal adenomas had statistically significant correlation with the presence of NASH (p=0.044, OR=3.48 (95% IC: 1.07-12.057), higher waist circumference (p=0.027) and obesity (p=0.041). Multivariate regression analysis showed an independent association of colorectal adenomas only with NASH after adjustment for age, gender, body mass index, glucose intolerance and waist circumference (OR=9.52 (95% IC: 1.71-52.85; p=0.010).

Out of 100 patients included in the study, 12 had advanced colorectal neoplasms (12%). Out of these, 10 were in the NAFLD group and only two in the control group. The prevalence of advanced colorectal neoplasm was 20% in the NAFLD group and 4% in the control group. The p value was statistically significant between groups (H² test X²=6.601, df=1, p=0.014>0.05; OR=6.000 (95% IC: 1.24-28.98) (table nr.2).

In the NAFLD group, the risk of colorectal advanced neoplasms had no statistically significant correlation with the presence of NASH (p=1.00). A multivariate regression analysis could not be done because of the small numbers of advanced neoplasms.

DISCUSSIONS
The definition of NAFLD requires that there is evidence of hepatic steatosis, either by imaging or by histology and there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatoegenic medication or hereditary disorders, etc. [10].

In the majority of patients, NAFLD is associated with metabolic risk factors such as obesity, diabetes mellitus, and dyslipidemia. NAFLD is histologically further categorized into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) [10].

The association of liver steatosis and the high risk of colorectal adenomas and colorectal cancer is still under assessment. The exact mechanism is yet unknown, but probably insulin resistance, with or without hyperinsulenia, and systemic inflammation are involved.

The present study is the first prospective case-control study, which comprised 50 patients with NAFLD (in 16 patients proven by liver biopsy) and 50 patients without NAFLD, in which panchromoendoscopy was carried out in order to detect more colorectal adenomas and carcinomas. We detected a 32% prevalence of adenomas in NAFLD vs. 22% in the control group (p=0.260) and a 20% prevalence of advanced neoplasms in the NAFLD group vs. 4% in the control group (p=0.014). Our findings are in agreement with previous studies. Recent investigations have demonstrated that NAFLD is an independent risk factor for colorectal adenomas and
In our study, in the NAFLD group, patients with NASH had a higher prevalence of adenomas (p=0.044, OR 3.48). After adjustment according to demographic and metabolic factors, NASH remained associated with adenomas (adjusted OR 9.52). Wong et al showed similar results in a cross-sectional study. After adjustment according to demographic and metabolic factors, NASH remained associated with adenomas (adjusted OR 4.89 (95% CI: 2.04-11.70; p=0.005) and advanced neoplasms (OR 5.34; 95% CI: 1.92-14.84). Just like in our investigation, in the study conducted by Wong et al, those with only liver steatosis had a similar risk of developing adenomas or advanced adenomas with that of the healthy population [7].

In the NAFLD group the left distribution of adenomas and advanced neoplasms was predominant, but without significant differences between groups. This is not in concordance with the literature, where an increased incident of right lesions was detected in patients with NAFLD [6,7]. We selected the chromoendoscopy, a priori, for a better detection of right colonic lesions. Panchromocolonoscopy failed to demonstrate a detection of more numbers of proximal adenomas than distal adenomas in patients with biopsy-proven NAFLD compared with those without NAFLD [11].

CONCLUSIONS
Patients with NAFLD have an high risk to develop advanced colorectal carcinomas than controls. In patients with NASH, the risk of colorectal adenomas is statistically and significantly higher than in patients with simple steatosis, after the adjustment according to demographic and metabolic factors. For an early colorectal cancer screening, we can select a group of high risk subjects.

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