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The Distribution of Liver Steatosis, Fibrosis, Steatohepatitis and Inflammation Activity in Alcoholics According to FibroMax Test

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. The diagnosis of alcoholic liver diseases is based on the history of alcohol abuse, clinical evidence of liver disease and laboratory abnormalities. The new non-invasive biomarkers have higher sensitivity to quantify and predict steatosis and fibrosis than ultrasonography.
Objectives. The aim of this study was to evaluate the prevalence of liver diseases in alcoholics by means of FibroMax.
Material and Methods. A total of 142 consecutive alcoholics were enrolled in the study. The prevalence of liver diseases was assayed by means of non-invasive biomarkers: fibrosis by FibroTest, steatosis by SteatoTest, steatohapatitis by AshTest (alcoholic origin) and NashTest (non-alcoholic origin) and necroinflammatory activity by ActiTest.
Results. 38.7% of alcoholics do not have fibrosis, 38% – steatosis, 94.1% – alcoholic steatohepatitis, 56.6% – non-alcoholic steatohepatitis and 33.6% – necroinflammatory activity. The insignificant fibrosis ($F < 2$) is present in 37.2%, advanced ($F \geq 2$) – 15.3% and cirrhosis (F4) – in 8.8%. Insignificant steatosis ($S < 2$) is observed in 31.3% and advanced ($S \geq 2$) in 30.5%. Minimal alcoholic steatohepatitis (H1) exists in 5.2% patients, moderate (H2) in none of the patient and severe (H3) in only one patient (0.7%). The distribution of NashTest scores is as following: N0 – 56.6%, N1 – 38.2% and N2 – 5.1%. Insignificant inflammatory activity ($A < 2$) is present in 40.8% of alcoholic patients but significant ($A \geq 2$) in 25.5%. The frequency of severe steatosis (F3) and necroinflammatory activity (A3) in patients with cirrhosis (F4) is 50% for each of them.
Conclusions. The prevalence of advanced fibrosis and cirrhosis evaluated by means of FibroMax in alcoholics is higher than in alcoholic liver disease (ALD) and lower than in mixed, alcoholic and non-alcoholic ones. This may indicate the presence of non-alcoholic liver disease in alcoholics (**Adv Clin Exp Med 2015, 24, 5, 823–827**).

Key words: non-invasive biomarkers, alcoholic liver diseases, FibroMax.

The treatment of alcoholic liver diseases (ALD) focused on the psychological and behavioral effects of alcohol consumption should also affect severe medical complications such as liver cirrhosis and also precursor lesions that presage cirrhosis such as perivenular fibrosis. The detection of precursor lesions facilitates the early intervention that prevents irreversible liver damage. Therefore, the evaluation of the degree of liver steatosis, fibrosis, and steatohepatitis has a fundamental importance for the therapeutic decision for patients with alcoholic liver diseases. Liver biopsy, that is considered the gold standard for evaluating the stage of liver fibrosis, is an invasive procedure and leads to complications in 0.6–5.0% of patients [1, 2]. Additionally, the liver samples from patients with chronic hepatitis C infection can be a cause of diagnostic error in up to 30% of patients, leading to an underestimation of the stage of liver fibrosis [3].

The gold standard for diagnosis of liver fibrosis – biopsy – is not recommended for the alcohol-

-dependent patients with symptoms of alcoholic liver disease. An accurate measurement of fibrosis as well as of the inflammatory activity allows simple noninvasive biomarkers developed by BioPredictive (Paris, France). FibroTest allows estimate liver fibrosis, SteatoTest – steatosis of different origins, AshTest – alcoholic steatohepatitis (ASH), NashTest – non-alcoholic steatohepatitis (NASH) and ActiTest – necroinflammatory activity. FibroMax is the association of these five tests on the same result sheet. In this study we would like to evaluate the distribution of the results of FibroMax test in alcohol dependent patients because at the same time it enables the exploration of steatosis, fibrosis, steatohepatitis and inflammatory activity.

Material and Methods

Subjects

The tested group consisted of 142 alcohol-dependent patients (mean: 46, range: 24–78, men – 127, women – 15) from detoxification ward (Department of Detoxification, Psychiatric Hospital in Choroszcz). Patients were initially examined and interviewed regarding their history of disease and their use of alcohol, drugs and smoking. The diagnosis of dependency was made on the basis of ICD-10 criteria (World Health Organization, 1992). The self-reported mean alcohol consumption was 1311 g of ethanol per week (range: 224–3318) and mean time of dependency was 18 years (range: 1–44). The time after cessation of drinking was between 0 and 2 days. Serum samples were collected from a peripheral vein once from each patient, immediately after admittance, before treatment. The sera were separated into 2 tubes and stored at –86°C until assayed. The research protocol was approved by the Bioethical Committee working at the Medical University in Białystok (Approval No. R-I-002/97/2012).

FibroMax Score

The serum biochemical markers: α2-macroglobulin, haptoglobin, apolipoprotein A1, γ-glutamyltransferase, alanine and aspartate aminotransferase, total bilirubin, fasting glucose, cholesterol and triglycerides were determined according to methods recommended by the provider of FibroMax – BioPredictive (Paris, France). Patients' data like age, gender, birth date, weight, height, and sample date, were required to generate the fibrosis, steatosis, and inflammation stages in the liver. FibroMax scores were computed by BioPredictive company according to the arrangement, and the results were provided with security algorithms, which excluded high risk profiles of false positive and false negative.

Determination of CDT by Direct Immunoassay (N Latex)

CDT, as the percentage of total transferrin (%CDT), was assayed by particle-enhanced imunononephelometry using N Latex CDT test (Siemens Healthcare Diagnostics, Marburg, Germany) on BN II System (Siemens Healthcare Diagnostics, USA). The reference interval of %CDT values ranged from 1.19 to 2.47% (1st to 99th percentile). The concentration of transferrin (reference interval: 2.0–3.6 g/L) was determined by the immunonephelometry with specific antibodies (N Antisera to human transferrin and haptoglobin) (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) on BN II System. The results were expressed in absolute (mg/L) and relative units (% of total transferrin concentration).

Statistical Analysis

Results were expressed as a mean and standard deviations. To allow comparisons between scores ANOVA rank test was performed. To calculate the correlation between variables Spearman's rank correlation coefficient was used. P values less than 0.05 were considered significant.

Results

The Prevalence of Liver Disease According to FibroMax Test

The following distribution of FibroTest scores (Fig. 1) was observed: F0 (no fibrosis) in 53 of 137 patients (38.7%), F0–F1 in 15 patients (10.9%), F1 (portal fibrosis) in 8 patients (5.8%), F1–F2 in 28 patients (20.4%), F2 (few septa) in 11 patients (8.0%), F3 (numerous septa) in 8 patients (5.8%), F3–F4 in 2 patients (1.5%) and F4 (cirrhosis) in 12 of 137 patients (8.8%). Summary, the prevalence of insignificant fibrosis ($F < 2$) was 37.2% (51 of 137 patients), advanced fibrosis ($F \geq 2$) – 15.3% (21 of 137 patients) and cirrhosis (F4) – 8.8% (12 of 137 patients). According to criteria given by BioPredictive the advanced fibrosis was presumed when FibroTest value was greater than 0.48. This is in 33 of 137 patients (24.1% what is the sum of 15.3% and 8.8%).

The distribution of SteatoTest scores was as following (Fig. 1): S0 (no steatosis) – 38.0% (51 of 134 patients), S0–S1 – 13.4% (18 of 134 patients), S1 (minimal steatosis) – 8.2% (11 of 134 patients),

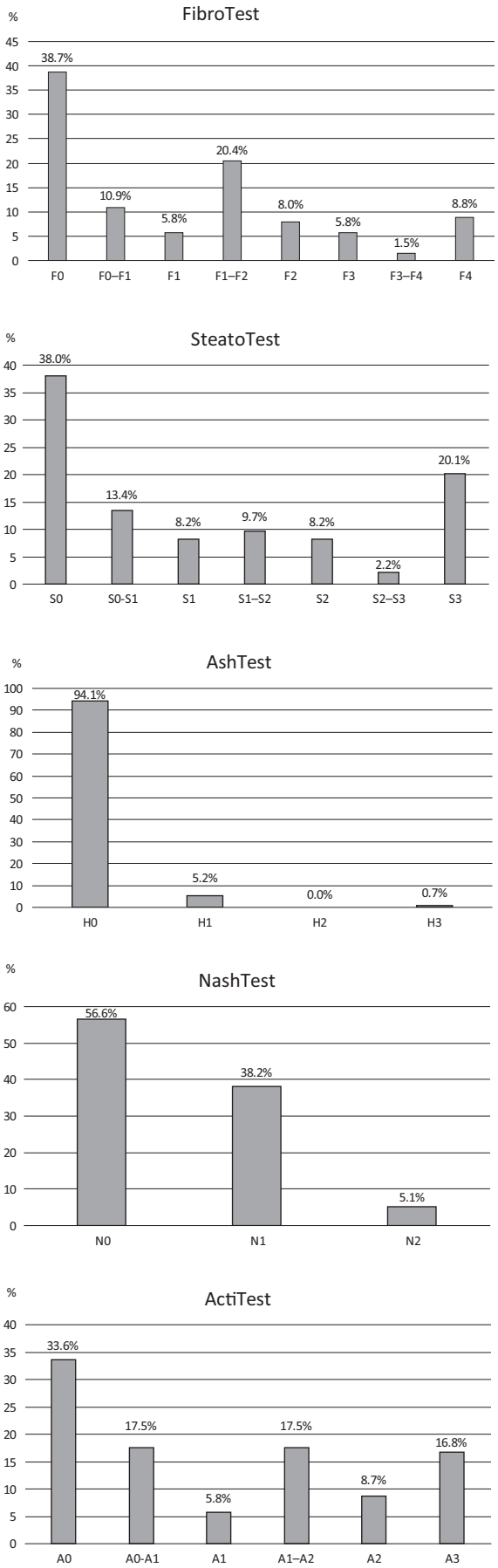

Fig. 1. The prevalence of fibrosis, steatosis, steatohepatitis and necroinflammatory activity in alcoholic patients

S1–S2 – 9.7% (13 of 134 patients), S2 (moderate steatosis) – 8.2% (11 of 134 patients), S2–S3 – 2.2% (3 of 134 patients) and S3 (advanced steatosis) – 20.1% (27 of 134 patients). Summary, insignificant steatosis (S < 2) was observed in 31.3% (42 of 134 patients) and advanced steatosis (S ≥ 2) in 30.5% (41 of 134 patients).

The following distribution of AshTest scores (Fig. 1) was observed: H0 (no hepatitis) in 127 of 135 patients (94.1%), H1 (minimal hepatitis) in 7 of 135 patients (5.2%), H2 (moderate hepatitis) in none of patient (0.0%) and H3 (severe hepatitis) in 1 of 135 patients (0.7%).

The distribution of ActiTest score (Fig. 1) of A0 was 33.6% (46 of 137 patients), A0–A1 – 17.5% (24 of 137), A1 – 5.8% (8 of 137), A1–A2 – 17.5% (24 of 137), A2 – 8.7% (12 of 137) and A3 – 16.8% (23 of 137). Thus, insignificant inflammatory activity (A < 2) was observed in 40.8% of alcoholic patients but significant (A ≥ 2) in 25.5% of patients.

The distribution of NashTest score (Fig. 1) was as following: N0 – 56.6% (77 of 136 patients), N1 – 38.2% (52 of 136) and N2 – 5.1% (7 of 136).

In patients with advanced steatosis (S ≥ 2, n = 41), the prevalence of minimal fibrosis (F < 2) was 51.2% (21 of 41 patients), advanced fibrosis (F ≥ 2) – 17.1% (7 of 41 patients), and cirrhosis (F4) – 19.5% (8 of 41 patients). In such patients the prevalence of minimal steatohepatitis (H1) was 12.2% (5 of 41 patients) and only 1 patient (2.4%) had severe steatohepatitis. Most patients with significant steatosis did not have steatohepatitis (35 of 41 patients, 85.4%). In patients with significant steatosis, the prevalence of minimal necroinflammatory activity (A < 2) was 41.4% (17 of 41 patients), and the prevalence of significant activity (moderate and severe) was also 41.4%.

The frequency of severe steatosis (F3) and necroinflammatory activity (A3) in patients with cirrhosis (F4) was 50% for each of them. Sixty seven percent of patients with cirrhosis no had steatohepatitis (H0).

The Correlation of FibroMax Scores with %CDT

%CDT results correlated with the results of NashTest (R = –0.224; p = 0.008) and ActiTest (R = 0.191; p = 0.025) but did not correlate with the results of FibroTest (p = 0.122), SteatoTest (p = 0.852), and AshTest (p = 0.073).

Discussion

The diagnosis of alcoholic liver disease is based on the following features: history of alcohol abuse, clinical evidence of liver disease and the results of

laboratory tests. The presence of steatosis, fibrosis, steatohepatitis and necroinflammatory activity can be evaluated by simple non-invasive biomarkers whose utility can be compared with the liver biopsy. These include SteatoTest, FibroTest, AshTest, NashTest and ActiTest, grouped in the one big test named FibroMax (BioPredictive, Paris, France). According to our results, 38.7% of alcoholics do not have fibrosis, 38% steatosis, 33.6% necroinflammatory activity, 94.1% alcoholic steatohepatitis and 56.6% non-alcoholic steatohepatitis. The prevalence of insignificant fibrosis is 37.2%, steatosis – 31.1%, necroinflammatory activity – 40.8%, non-alcoholic steatohepatis – 38.2% and alcoholic steatohepatitis only 5.2%. Advanced fibrosis is observed in 15.3% of patients, cirrhosis in 8.8%, steatosis in 30.5%, necroinflammatory activity in 25.5% and non-alcoholic steatohepatitis in 5.1% and significant alcoholic steatohepatitis in only 0.7% of patients. In summary, fibrosis is present in 52.5%, steatosis in 61.8%, necroinflammatory activity in 76.3%, non-alcoholic steatohepatitis in 43.3% and alcoholic steatohepatitis in 5.9% of alcoholics. For comparison, the following prevalence of liver diseases among drinking patients were obtained by others: up to 90% of alcoholics have fatty liver, 5–15% of them have alcoholic steatohepatitis and cirrhosis, and from 10 to 35% of them exhibit alcoholic hepatitis [4, 5].

There is evidence that in patients with alcoholic liver disease, the presence of steatosis is associated with the progression of fibrosis and with necroinflammatory lesions [6, 7]. The prevalence of advanced fibrosis and high necroinflammatory activity in alcoholic patients with advanced steatosis reaches the values of 17.1% and 41.4%, respectively. Interestingly, most patients with advanced steatosis (85.4%) do not have alcoholic steatohepatitis. Although necrosis and inflammation are the precursors of fibrosis (transformation of stellate cells into fibrous tissue), the cirrhosis commonly develops without overt alcoholic hepatitis [8]. The frequency of an advanced stage of steatosis (S3 – 20.1%) and cirrhosis (F4 – 8.8%) was much higher than the frequency of severe alcoholic steatohepatitis (H3 – 0.7%). The prevalence of ASH and NASH in the general population is not known because of the impossibility of performing liver biopsy, but it is known that about 10–35% of alcoholics exhibit alcoholic hepatitis [9]. The incidence of alcoholic steatohepatitis in our study is only 5.9%, but 43.3% of alcoholics exhibit presumed non-alcoholic steatohepatitis.

Staging of chronic liver disease, especially liver fibrosis, is essential to define prognosis and management of disease. According to METAVIR classification, fibrosis is defined as two stages that significantly modify the management of liver disease. There is advanced fibrosis which is defined as $F \geq 2$, and cirrhosis defined as F4. In our alcohol abuse patients without confirmed alcoholic liver disease, the prevalence of presumed advanced fibrosis (defined as $F \geq 2$ in FibroTest) is 24.1%. Taking into account the fact that about 30–35% of heavy drinkers develop advanced forms of ALD such as advanced fibrosis and cirrhosis, the discrepancy between these outcomes is apparent [10]. Thus, using evaluation of fibrosis by FibroTest, about 5–10% of alcoholics do not have diagnosed advanced fibrosis. It is extraordinary because the prevalence of presumed fibrosis in the general population, estimated by FibroTest, is 2-times greater (2.8%) than the prevalence of confirmed fibrosis 1.3% [11]. The prevalence of presumed and confirmed fibrosis in alcoholic liver disease is established at 7% and 8%, but combined, alcoholic and non-alcoholic liver disease, the prevalence of presumed fibrosis is 29% and confirmed fibrosis is 42% [11]. Thus, when alcoholic liver disease coexists with non-alcoholic, the prevalence of presumed fibrosis evaluated by FibroTest is underestimated but when alcoholic liver disease exists alone, the prevalence of presumed fibrosis are in agreement with the prevalence of confirmed disease. In another study, the advanced fibrosis (F2–F4) was present in 63% of patients with chronic alcoholic liver disease by biopsy examination [7]. Our result of 24.1% is located between 7% and 29%, which can indicate that patients do not have only alcoholic liver disease, but combined with non-alcoholic. This suspicion might be substantiated by the fact that 43.3% of alcoholics have presumed non-alcoholic steatohepatitis (N1 and N2 score in NashTest). Additionally, the results of the correlation study showed that the staging of fibrosis, and also steatosis and alcoholic steatohepatitis, are not associated with the alcohol consumption marker – CDT (this does not correlate with %CDT). Contrarily, %CDT correlates with necroinflammatory activity and non-alcoholic steatohepatitis, which confirms the inclusion of metabolic risk factors in alcoholic liver disease.

In summary, the prevalence of liver diseases in alcoholics estimated by means of FibroMax Test is higher than the prevalence of alcoholic liver disease confirmed by biopsy, which could indicate the overestimation of liver disease occurrence recognized by FibroMax Test or on the presence of factors other than alcohol involved in the progression of liver disease.

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