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IMPACT OF ALCOHOL CONSUMPTION IN PATIENTS WITH LIVER CIRRHOSIS YOU ARE WAITING FOR A LIVER TRANSPLANT EPIDEMIOLOGY, CONSUMPTION VERSUS ABSTINENCE, NUTRITIONAL STATUS AND SURVIVAL

-SUMMARY OF THE DOCTORAL THESIS-

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List of abbreviations

ADH1B	gene involved in alcohol metabolism
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
ALDH2	gene involved in alcohol metabolism
ALT	Alanine-aminotransferase
AST	Aspartate-aminotransferase
AUDIT	Alcohol Use Disorders Identification Test
AUDIT-C	Alcohol Use Disorders Identification Test-Consumption
BMI	Body mass index
CAGE	Cut, Annoyed, Guilty, Eye-opener
CDT	Carbohydrates Deficient Transferrin
CoA	Coenzyme A
CT	Computer Tomograph
CYP2E1	cytochrome P450 2E1
DASS	Depression, Anxiety, Stress Score
ESG	Global Subjective Assessment Questionnaire
ESPEN	The European Society for Clinical Nutrition
EtG	Ethyl-glucuronide
EtS	Ethyl sulfate
FAEE	Ethyl Esters of Fatty Acids
GGT	Gamma-glutamyl-transpeptidase
HCC	Hepatocellular carcinoma
MAC	Median Arm Circumference
MCV	Mean corpuscular volume
MELD	Model for End-Stage Liver Disease (Score)
NAD	Nicotinamide-adenine-nucleotide
NADH	Nicotinamide-adenine-nucleotide reduced form
NKC	Natural killer cells
PEth	Phosphatidyl-ethanol
PMI	Psoas Muscle Index
MRI	Nuclear Magnetic Resonance SMI Skeletal Muscular Index
TSF	Triceps skin fold
WHO	World Health Organization

GENERAL SECTION

Global data on alcohol consumption showed that in 2019 it was the cause of 2.6 million deaths worldwide, which meant 4.7% of all deaths. It is estimated that 400 million people worldwide suffer from alcohol-related diseases and another 209 million are dependent on alcohol. In Europe, in the same year, the highest levels of total alcohol consumption were recorded, at 9.2 liters per capita per year.

The highest mortality comes from cardiovascular diseases caused by alcohol consumption and is followed by mortality from gastrointestinal and liver causes, alcoholic liver cirrhosis [1]. There is no scientific evidence that there is a minimal amount of alcohol consumed that does not present any health risk [2].

The national data, communicated by EUROSTAT, show that in 2019, 35% of Romanians declared excessive alcohol consumption at least once a month, this represents episodic excessive consumption. For the year 2023, a total average consumption of alcoholic beverages is reported to increase compared to previous years, which reached 10.49 liters of pure alcohol/capita/year.

The same statistics show that, taking into account the duration of abstinence, there are more women who have never consumed alcohol than men (19.9% vs. 6.6%), or have stopped drinking for over a year (24.7% vs. . 13.4%), or have consumed alcohol in the last year (44.5% vs. 19.9%) [1]. And global data shows that men consume more alcohol than women worldwide. More alcohol is consumed in developed countries than in developing countries. Increased rates of alcohol dependence are more common in men, and higher amounts of alcohol are consumed in communities with lower socioeconomic status. The environment and family have the greatest influence on the behavior related to alcohol consumption [3], [4].

The determining factors for alcohol consumption are individual factors, of a psychological nature, the ability to adapt to stress and discrimination, with a negative impact on the individual, and alcohol consumption becomes a means of escape. Genetic factors represent, another group, with a 50% risk of alcohol consumption disorder [5]. Environmental and social factors are most important in the development of alcohol use disorders. The number of relatives who consume alcohol increases the chance of early onset of alcohol dependence [6]. Other risk factors have been found to be a disorganized family environment and social structure with unhealthy habits. Unemployment and social isolation, low economic-financial status and a minimum level of education were associated with alcohol dependence and abuse.

Community factors are represented by advertising, availability and ease of procuring alcohol.

Once alcohol consumption begins, the problem arises of stopping and achieving prolonged abstinence, which is the main therapy for all patients with liver disease caused by alcohol consumption. In order to achieve this goal, both the doctor and the psychologist have an important role, by clearly communicating the negative effects of alcohol consumption depending on its quantity and frequency. Abstinence from alcohol consumption is the only factor that improves the prognosis of alcohol-induced liver diseases. The curative treatment of liver cirrhosis is liver transplantation, and the 6-month pre-transplant abstinence rule is only a condition for listing but is not predictive of relapse [7] and applies to patients with alcohol-induced cirrhosis.

The standardization of the amount of alcohol contained in different drinks required the development of an international convention in which the pure alcohol (in grams) contained in each alcoholic drink is reported, this being called a "standard drink". For Romania, the "standard drink" is set at 12 g. It is difficult for alcohol drinkers to self-estimate their consumption, because patients are often not able to remember the different types of drinks and their quantity [8]. Chronic consumption in men of more than 2 standard drinks per day and in women of more than 1 standard drink per day increases the risk of liver damage.

Individual consumption, depending on the amount of alcohol, varies from a consumption considered safe for the healthy population (1 "standard drink"/day for women and 2/day for men), to an excessive consumption, above these amounts, reaching up to at risky or dangerous consumption (> 8 "standard drinks"/day for men and > 6 for women) [9].

A clinical examination needs to be completed with the application of self-assessment questionnaires for alcohol consumption at a risky or harmful level, even more so in the presence of liver diseases due to alcohol consumption. This evaluation should be performed in all patients who show signs of alcohol abuse [10] but also in those with a normal clinical examination. The WHO recommends the AUDIT questionnaire which is composed of 10 questions, which refer to the frequency of consumption, the amount of alcohol consumed expressed in "standard drinks", the symptoms of addiction and feelings of guilt or remorse. All of this information is based on the honesty of the respondents, with a margin of error due to intentional or unintentional underreporting. Shorter questionnaires are more attractive so the AUDIT-C (concise identification test) includes the first 3 questions of the AUDIT, has been validated for

identification alcohol abuse, with a sensitivity of 84-86%, at a threshold score of 4 points for men and 3 points for women out of a maximum score of 12 points.

Another simple questionnaire is the 4-question CAGE test, which identifies alcohol use disorders and alcohol dependence and assesses behavior associated with awareness of alcohol misuse as well as symptoms of dependence syndrome. The threshold value, from which the probability of risky alcohol consumption increases, is 2 affirmative answers.

The FAST Rapid Screening Test for the detection of harmful alcohol consumption and addiction is composed of 4 questions also extracted from the AUDIT test. The threshold for harmful alcohol consumption is 3 points [11]. These tests provide the first information on the level of ethanol consumption of patients and in our study of cirrhotic patients.

According to WHO data from 2018, alcohol, globally, would be responsible for 3 million deaths, representing 5.3% of all deaths that would be due to harmful consumption. Abstinence or infrequent alcohol consumption increases the chance of survival by 1.6 times compared to more frequent alcohol consumption [12].

The epidemiology of alcoholic liver disease shows that early forms of alcoholic liver disease are often undiagnosed because clinical manifestations are absent and patients are asymptomatic for a long time. A daily alcohol consumption not associated with meals has a 2.5 times higher relative risk of developing cirrhosis, while the incidence of cirrhosis decreases if alcohol is associated with food intake. The amount of alcohol consumed is the main risk factor for alcoholic liver disease, although only 20-30% will develop steatohepatitis and after about 10 years, 10% reach the stage of liver cirrhosis [9]. Other factors influencing progression to liver disease are consumption pattern, predominant type of alcoholic beverage, presence of malnutrition or obesity, association with chronic B or C viral infections, and genetic factors [13].

Chronic liver damage due to alcohol consumption is because alcohol is metabolized mostly in the liver. Absorbed from the intestine freely passing through biological membranes after absorption, alcohol is freely transported into the plasma. A part is eliminated renally after undergoing a biotransformation through glucuronidation and sulfation, thus resulting in the products ethyl-glucuronide (EtG) and ethyl-sulfate (EtS) which can be dosed in blood or urine as a marker for alcohol consumption. The oxidative pathway by which most ethanol is metabolized in the liver. The metabolism of alcohol produces acetaldehyde, a toxic product that contributes to cellular damage [14]. Nicotinamide-adenine-nucleotide (NAD) which from the form reduced NADH is reoxidized and slows the metabolism of alcohol and causes the

symptoms of alcohol intoxication. Cytochrome P450 (CYP2E1), contributes to the oxidation of alcohol, having an important role in metabolizing high concentrations of alcohol. Catalase, has an important role in the brain, along with CYP2E1 in the production of acetaldehyde. Acetaldehyde is part of the category 2 carcinogenic products and turns into acetic acid. Acetaldehyde does not cross the blood-brain barrier and thus remains and is distributed in the central and peripheral nervous system [15].

The non-oxidative pathway metabolizes small amounts of ingested alcohol with the formation of esters, which may have pathological and diagnostic relevance. This pathway is activated after the first oxidative pathway is inhibited [16]. Under the action of ethyl ester synthetase, fatty acids are esterified into fatty acid ethyl esters that persist for at least 24 hours after the alcohol has been eliminated from the body. Phosphatidyl-ethanol (PEth) results from phospholipids is poorly metabolized and can accumulate to detectable levels following chronic consumption of large amounts of alcohol. These two minor metabolites can be used as biomarkers for alcohol consumption.

The effects of alcohol on the body are manifested by toxicity that is directly due to alcohol and some metabolism products, acetaldehyde, NADH, acetylCoA, FAEE, PEth. On the nervous system, alcohol has both a stimulating effect, in the initial phase, and a sedative effect by decreasing cortical activity and glucose metabolism at this level [17]. Alcohol produces changes on the liver from minor to advanced fibrosis (cirrhosis) because toxic compounds are produced through metabolism that cause liver damage, which fall into three types of liver diseases. The metabolic products of the oxidative pathway produce hepatic steatosis, followed by inflammation at the hepatocytic level that translates into alcoholic hepatitis and finally the replacement of liver tissue with fibrous tissue represented by the cirrhotic stage.

Alcohol becomes the main etiology of cirrhosis globally as of 2018 when it is estimated that 28 to 50% of decompensated cirrhosis is caused by alcohol consumption [1]. In Europe, alcoholic cirrhosis has a prevalence of 16-78%, where it is associated with a high level of consumption. In the American population they demonstrated increases in the percentage of cirrhosis associated with alcohol of 0.8% per year [18]. The risk of alcohol consumption in advanced fibrosis liver disease is high and alcohol intake should be discouraged, based on clinical evidence that cessation of alcohol consumption at any point in the natural history of liver disease reduces this risk of progression and complications [19]. Continued alcohol consumption in those with an alcoholic liver disease causes decompensation with the onset of ascites and the appearance of complications, variceal hemorrhage or hepatic encephalopathy,

leading finally to death. Alcohol consumed in large quantities and with increased frequency was distantly associated with increased values of biomarkers especially gamma-glutamyl-transpeptidase (GGT) and death,

Global mortality among alcoholic cirrhosis was 607 thousand deaths in 2016, in Western Europe in the short term, 1 year [20], it is 25-30%. and at 5 years mortality was reported at 41% [21]. In Romania, in 2010 there were 8.1 deaths/100,000 inhabitants for men and 2.1/100,000 inhabitants for women [22].

Active drinkers frequently had a degree of malnutrition associated with sarcopenia. Alcohol is a drink rich in calories but these are "empty calories", they have no other nutrients associated with them and usually the drinks contain more calories than the body needs. Alcohol metabolism occurs not only in the liver and brain, but also in skeletal muscle by disrupting proteostasis. Alcohol consumption leads to the alteration of protein synthesis, after intestinal and postprandial absorption inducing the state of "anabolic resistance", which is defined by adequate intake of nutrients but suboptimal protein synthesis, especially at the skeletal muscle level. Malnutrition occurs in 20% of patients with well-compensated cirrhosis and up to 60% in patients with advanced decompensated disease. Sarcopenia secondary to malnutrition has a negative impact on quality of life and survival in the cirrhotic patient and affects up to 70% of decompensated cirrhosis.

The best options for assessing sarcopenia are: measuring grip strength, endurance and walking speed, the Subjective Global Assessment (ESG) questionnaire associated with anthropometric measurements (mid-1/3 arm circumference or triceps skinfold) are just a few. among the recommended evaluation methods. Other methods are radiological, computed tomography or magnetic resonance imaging to diagnose sarcopenia. Imaging examination of the cirrhotic patient awaiting transplantation is mandatory and consequently the psoas muscle area can be assessed in all these patients and the psoas muscle or skeletal index calculated.

Biomarkers have proven their usefulness over time in the diagnosis and confirmation of alcohol abuse. The classic ones for alcohol consumption are ALT & AST, GGT, MCV common laboratory tests that have been used to detect alcohol consumption. A new biomarker is CDT which in combination with GGT have been described as diagnostic for alcohol use disorders [85]. Direct biomarkers derived from ethanol metabolism directly measure alcohol levels. These are ethyl glucuronide (EtG) and ethyl sulfate (EtS), phosphatidyl ethanol (PEth) and fatty acid ethyl esters (FAEE). [23]. Being metabolites of alcohol, they are not influenced by comorbid conditions and therefore have less chance of false positive or negative values [24].

Alcohol consumption is due to an accumulation of individual and social factors that interact and determine this behavior. Depression, anxiety, and stress are risk factors for alcohol-related diseases, and there is a significant association between alcohol dependence and these common mental disorders. The DASS 21 questionnaire is a useful tool for assessing these emotional states.

The only treatment with curative intent for these patients is liver transplantation. Abstinence from alcohol consumption can improve liver failure and increase the survival of these patients while awaiting transplantation. Liver transplantation in this case ensures a 90% survival at one year, in patients who in the absence of transplantation would have an estimated survival of 10% for the next year [25].

PERSONAL CONTRIBUTIONS

Working hypothesis and general objectives

In this study we focused on the causes of liver cirrhosis, with an indication for liver transplantation, the existence of differences between etiologies and especially the etiology related to alcohol consumption, the demographic and epidemiological profile of alcohol consumption in the selected group of patients.

We wanted to develop a model of alcohol consumption in cirrhotic patients, quantity, frequency and dependence, active consumption and abstinence and a diagnosis as complete as possible.

The usefulness of self-assessment questionnaires of alcohol consumption, for patients with multiple etiology of cirrhosis, including alcoholic, in determining the predominant cause in these patients.

There are variations in the nutritional status of cirrhotics, depending on the etiology of the liver disease, the age of the patient and the MELD (Model for End-Stage Liver Disease) score. Usefulness of anthropometric parameters and direct imaging assessment of the psoas muscle to assess sarcopenia.

The utility of indirect and direct markers in monitoring recent alcohol consumption for patients with alcoholic cirrhosis in order to be included on the waiting list for liver transplantation.

Survival on the waiting list and liver transplantation as an end event in the first year of inclusion.

OBJECTIVES

The main aim of the paper is to identify the level of alcohol consumption in patients with liver cirrhosis admitted to be listed. To assess the correlation of alcohol quantity and frequency of consumption with etiology of cirrhosis, nutritional status, and survival stratified by etiology of liver disease. Utility of direct biomarkers for identifying recent use and indirect for magnitude of use effects and adherence to abstinence.

Achieving these goals could help us in the more complex approach to the patient with cirrhosis caused by alcohol consumption, paying increased attention to the correction of nutritional deficits, achieving and maintaining abstinence tested at different time intervals, psychological interventions and relapse prevention during the patient's it is waiting for the transplant but also after the transplant and last but not least the prioritization of them as recipients. Arresting muscle loss and recovery in sarcopenic patients to increase survival both before and after liver transplantation.

General research methodology

We performed four prospective, cohort, observational studies that consecutively included subjects with liver cirrhosis, at the first admission in the Gastroenterology, Hepatology and Liver Transplant Clinic of the Fundeni Clinical Institute, to be evaluated for listing, starting in June 2015.

Inclusion criteria for the first study

Inclusion period: June 2015 – May 2018, adult patients over 18 years of age, who presented themselves for the first time in our clinic, to be considered candidates for liver transplantation.

The presence of liver cirrhosis as the main condition, with different etiologies, decompensated or not, complicated or not. Among the tumor complications, superimposed hepatocellular carcinoma (HCC) meeting the Milan criteria.

Patients who agreed by signing consent to participate in the study and to answer the questions in the self-report questionnaires of alcohol consumption.

Exclusion criteria:

Patients with alcoholic hepatitis superimposed or not on liver cirrhosis, with HCC outside the Milan criteria, hepatic encephalopathy due to the impossibility of answering the questionnaire, who did not agree to participate and did not sign the consent.

The cohort consisted of 175 patients who were followed for one year, at 0, 1, 3, 6, and 12 months for the final event of death, transplant, or pending.

Inclusion criteria in the second study

From the group of patients in study 1, we selected those who performed the computed tomography (CT) or nuclear magnetic resonance (NMR) imaging examination, as part of the mandatory balance, in the first 6 weeks after admission, with the measurement of the area of the psoas muscles left and right at the level of the L3 vertebra or at the level of the L3-L4 intervertebral space and were evaluated anthropometrically: arm circumference in the middle 1/3 (MAC), skinfold at the level of the triceps brachii (TSF), height, weight, body mass index (BMI)

We excluded patients who did not have these measurements performed or had incomplete data.

The cohort was 133 patients who were followed until the final event of death, transplant, or pending.

Third study inclusion criteria

In the third study, patients were included between November 2023 and May 2024, with cirrhosis of the liver linked to alcohol consumption as a single or combined cause, from whom blood samples were collected from which direct and indirect markers for alcohol consumption were analyzed. alcohol. They agreed to answer the questionnaire for depression, anxiety and stress (DASS).

This third study included 54 selected patients with ethanolic and/or combined cirrhosis.

The fourth study inclusion criteria

In this study, we used the cohort from the first study and the one from the second study with the same inclusion criteria for the evaluation of mortality on the waiting list and the transplant end-point.

Study 1. Usefulness of the Three Alcohol Assessment Questionnaires and Indirect Biomarkers in Alcohol Prediction of Combined Cause Liver Cirrhosis

INTRODUCTION: To characterize alcohol consumption among patients with liver cirrhosis to be evaluated for liver transplantation and to outline their epidemiological and demographic profile. Let's highlight the differences between subjects who consume amounts at risk of alcohol or are addicted and those who have never consumed alcohol or had a rational consumption. Let's compare subjects who stopped drinking at least 12 months ago with those who drank alcohol until recently and who were among those who should be given the 6-month abstinence rule before being considered a transplant candidate.

How can we quickly and accurately assess the cirrhotic patient for the alcoholic component of liver damage produced by this commonly encountered toxin.

PATIENTS AND METHODS: The evaluation of alcohol consumption was carried out with the help of the clinical interview and 3 short screening questionnaires that were applied to the psychologist specializing in addiction. We chose the CAGE, AUDIT-C and FAST questionnaires because they are short with few questions but of major importance in detecting alcohol abuse. The CAGE questionnaire is composed of 4 questions. A score above 2 raises the suspicion of the presence of alcohol dependence. It is not a diagnostic tool but only a good first indicator that the evaluation should be continued [26].

Another short questionnaire is the AUDIT-C, a 3-question questionnaire validated for alcohol abuse. With a score between 0 and 12 points, the threshold for alcohol abuse is 3 points for women and 4 points for men. Each question has answers marked from 0 to 4 points [27]

The third questionnaire, the FAST quick detection test of the harmful effects of alcohol consumption, and is composed of 4 questions from the AUDIT test. It has been prepared for use in emergencies and is considered positive at a total score of ≥ 3 points. All answers are marked from 0 to 4 points, the maximum score being 16 points [28]. The patients were divided into three groups according to the hospitalization diagnosis and alcohol consumption: cirrhosis due exclusively to alcohol consumption, viral liver cirrhosis or another cause associated with alcohol consumption, and liver cirrhosis without alcoholic cause. The second set of possible

predictive factors for alcoholic etiology, we included indirect biomarkers, gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and their AST/ALT ratio, mean corpuscular volume of erythrocytes (MCV). We also associated the liver disease severity score with MELD-Na sodium being a score calculated based on serum laboratory tests that evaluate liver function: creatinine, total bilirubin, INR and sodium.

RESULTS AND DISCUSSIONS: In this study mixed etiology prevailed, alcohol associated with virus or other causes, followed by alcoholic, the male gender was predominant with more than 70%, the average age in our group was 53 years, with a majority at 50 years, a sufficient time interval of alcohol consumption for the development of liver cirrhosis. Although most patients came from urban areas, the majority of alcohol consumers were from rural areas, 78.46%. The mixed etiology of cirrhosis represented by alcohol consumption superimposed on a viral or autoimmune cause, which we called combined/mixed etiology, represented 48% of the total, followed by 28.57% only alcohol consumption. The spectrum of mixed etiology was clearly dominated by alcohol consumption associated with previous exposure to hepatitis B virus (HBV) manifested by the presence of HBc antibodies with the absence of HBs antigen.

At the time of the first admission to the clinic, a percentage of 37.71% of those with alcoholic cirrhosis were not abstinent and declared alcohol consumption in the last 3 months prior to admission. They were considered active drinkers and their evaluation for transplantation was delayed by 6 months, during which some of the active drinkers recover their liver function and the need for transplantation is reduced [29-31]. Daily or almost daily consumption is declared by 1/3 of the patients, and among those with alcoholic cirrhosis 56% consume alcohol daily and 24% weekly, resulting in 80% chronic alcohol consumption. Similar figures were also obtained in patients with combined cirrhosis, more than 60% have chronic alcohol consumption.

The CAGE and AUDT-C scores divided by the threshold value for alcohol abuse can be used as independent predictive factors for the alcoholic etiology of cirrhosis with an accuracy of 83.5% for AUDIT-C and 76.9% for CAGE but also for the combined one they had an accuracy of 80% AUDIT-C and 72.8% CAGE, in a positive statistical correlation. The FAST questionnaire correlated negatively with alcohol exposure and was a significant predictor of nonalcoholic etiology. Multivariate analysis demonstrated that both CAGE and AUDIT-C tests are predictors for alcoholic etiology with a prediction accuracy of 83.5% and for the combined etiology these two tests had a prediction accuracy of 85.6%, with statistical significance. No

significant difference was demonstrated in the score of the questionnaires for the prediction of alcoholic etiology versus the combined, all three showed an approximately 1:1 chance.

We recommend CAGE and AUDIT-C be used both separately and in combination, both having a sensitivity of approximately 70% and a specificity of approximately 90%. We can combine the Indirect Marker Assessment Questionnaire score and the MELD-Na Liver Disease Severity Score to confirm alcohol use as the cause of liver disease. Indirect serum biomarkers for the detection and measurement of alcohol exposure in alcohol-induced liver disease are indicative and remain indirect markers with sensitivity between 24-86% and specificity 40-96% in agreement with previous studies [32-35]. We used biomarkers collected at baseline, administered the questionnaires, and calculated the MELD-Na score, to determine whether they are predictors of cirrhosis with alcohol exposure. Univariate analysis found significant differences for alcoholic etiology compared to nonalcoholic for ALT, AST negatively correlated and GGT and MELD-Na positively correlated with superunit OR, in multivariate analysis a negative correlation was obtained for ALT and positive correlation for GGT and score MELD-Na with supraunit OR. There was no significant difference between the other etiologies, although the combined etiology has an alcoholic component, but these biomarkers also increase in advanced liver disease from other causes. For the evaluated markers we calculated threshold values possibly associated with alcoholic etiology, ALT at 67 IU/l has a sensitivity of 46%, GGT at 67 IU/l has a sensitivity of 54% and for the MELD-Na score at 17.5 we obtained a sensitivity of 64%

CONCLUSIONS: The study provides information on the structure of the alcoholic cirrhotic population, on the frequency, amount and type of alcohol consumed. Since there are no significant differences between the scores of the questionnaires obtained by patients with only alcohol consumption and those with alcohol consumption and other causes, it leads us to consider that the initial approach of those with mixed etiology must be similar to those with alcoholic etiology. It is recommended that all patients on initial admission be given the CAGE and AUDIT-C evaluation tests from the first day of admission, without this topic having been addressed previously. It would be recommended that these tests be administered to patients by an expert addiction psychologist. Thus, patients with mixed etiology of cirrhosis and those with only alcohol as the cause will have to be included in the psychological counseling program for obtaining and maintaining abstinence required at least 6 months prior to inclusion on the waiting list.

Assessment of alcohol exposure based only on indirect biomarkers can have false positive values, and there are many pathological conditions in which elevated values are not related to alcohol consumption, especially in advanced, cholestatic liver diseases. From the presented analysis, indicative of recent alcohol consumption, a value of less than 67 for ALT and greater than 67 for GGT can be used in patients with alcoholic cirrhosis associated with a MELD-Na score above 17.5 points.

Study 2. Sarcopenia in the cirrhotic patient awaiting liver transplantation

INTRODUCTION: Approximately 50% of cirrhotics will reach this complication, especially in the decompensated stages [36]. Malnutrition is considered to be an important predictor for patient survival [37-38]. Malnutrition produces a progressive loss of muscle mass called sarcopenia. In the general population sarcopenia affects up to 30% of subjects, but in the cirrhotic population sarcopenia can reach up to 70% [39–40] and almost 100% will suffer from some degree of malnutrition before being transplanted [41]. Malnutrition is assessed by indirect methods: anthropometric measurements, easy to perform, with simple instruments. Direct methods are more expensive and require computed tomography (CT) or magnetic resonance imaging (MRI) evaluation with image acquisition at the level of the L3 vertebra and measurement of the psoas muscle area [42]. For the cirrhotic population, measuring the area of the psoas muscle and calculating the psoas muscle index is a more accessible method than in the general population because the assessment of the disease itself involves imaging. This muscle is not influenced by the presence of ascites, as are the abdominal girth muscles [40].

PATIENTS AND METHOD: The prospective observational study carried out between March 2015 and May 2018 evaluated 133 patients who presented for a pre-liver transplant check-up and underwent a CT or MRI examination within the next 4-6 weeks from the time of the first hospitalization, with a section at the level of the L3 vertebra. Measurements of psoas muscle area, anthropometric measurements, MAC, TSF were performed and were evaluated sonographically for the presence of ascites and its volume, and the calculated BMI was corrected for the presence of ascites. For the threshold value of PMI in the determination of sarcopenia, we used the data from the literature, values obtained on a healthy population, of living donors of the left liver lobe who performed a CT examination in the evaluation card for

donation, and a value of 4.62 cm2/ was obtained for men m2 and for women 2.66 cm2/m2. Although this study also has limitations due to the maximum donor age of 59 years, it is among the few studies that establish threshold values for sarcopenia in a healthy population [43].

RESULTS AND DISCUSSIONS: The incidence of sarcopenia is estimated to be between 5-13%, in the study by Stephan von Haehling, conducted on subjects from the United States, France and Italy. Another study from Germany shows that in the ward of geriatric patients sarcopenia has an incidence of 25% [44]. If these are the data for apparently healthy populations, the prevalence in the cirrhotic population is reported to be between 30 -70%, with these large variations due to different diagnostic tools and the stage of liver disease at which sarcopenia was diagnosed. The leaders in the field of sarcopenia Global Leadership in Sarcopenia (GLIS), in the Delphi Consensus 2024, aimed to develop a conceptual definition for sarcopenia and 6 general aspects of sarcopenia were accepted [45]:

- It is a generalized damage to the skeletal muscles

- There is a positive association between age and the presence of sarcopenia

- The definition of sarcopenia must not have differences between the ambulatory regime and hospitalization

- The definition of sarcopenia should not vary according to the patient's age and conditions

- The definition should be the same for the clinician and the researcher

- It is a disease with potential for improvement

In the study group, sarcopenia was present in 18% of patients and in 21.9% of those with alcoholic cirrhosis and 19.7% in the combined etiology, it predominated among male patients and the median age was 52 years. Another important factor in association with sarcopenia is the etiology of liver disease and alcoholic etiology has been shown to accelerate the progression of sarcopenia [46]. In our group, there were 2 times more sarcopenic patients in the group with alcoholic and mixed etiology than in the nonalcoholic group, although these differences did not reach statistical significance. An active consumption of alcohol at the beginning was accompanied by a decrease in muscle mass, 22% of them being sarcopenic compared to 15% of those who stopped drinking at least 6 months before. Hepatocarcinoma was not associated with sarcopenia, probably because the patients were selected in the Milan criteria and the tumor was not advanced. Minimal to voluminous ascites was significantly associated with sarcopenia.

The presence of sarcopenia in obese patients is another association highlighted in the literature, but in our group most sarcopenic patients had a normal BMI, significantly more compared to the obese. We believe that patients with a normal BMI should also be evaluated for the presence of sarcopenia, as these patients with a normal BMI have a 4.6 times greater chance of decreased muscle mass compared to the obese.

The anthropometric measurements performed in our study, which are easily obtained in clinical practice, showed a positive association in linear regression with the psoas muscle index calculated on the basis of imaging evaluations. The results show that anthropometric values do not have significant applicability in clinical practice, mainly due to the lower sensitivity and specificity in distinguishing between sarcopenic and nonsarcopenic, but for males they were significantly associated with sarcopenia below the threshold values of 25.5 cm MAC, 4 .75 cm TSF, 74.5 kg weight and 24.5 BMI. The MELD-Na score correlated significantly but negatively with the PMI at a threshold value of 17.5 points.

CONCLUSIONS: Sarcopenia is common among cirrhotic patients, aggravates the evolution and causes decompensation and complications, increases the duration of hospitalization and requires admission to intensive care more often than nonsarcopenic patients. Sarcopenia was not significantly associated with alcohol consumption and the etiology of cirrhosis, although they were 2 times more likely to be sarcopenic.

Even though in our study most subjects with sarcopenia fell into the group with normal BMI, anthropometric measurements can be performed in cirrhotic patients and using threshold values can guide us in which patients to perform psoas muscle assessment on CT or MRI images. This discrepancy with other studies shows us that there is an increased risk of sarcopenia in patients with normal BMI, who should not be neglected in the complex evaluation for sarcopenia. The MELD-Na score that replaced the simple MELD score in 2018 was not associated with sarcopenia in our study, but for the future it is possible in larger groups of patients that there is a correlation between sarcopenia, the MELD-Na score and mortality, so that they to receive additional points and be prioritized for transplantation.

Study 3. Utility of direct biomarkers in detecting recent alcohol use and association with common mental disorders

INTRODUCTION: The frequency of advanced liver disease associated with alcohol consumption is constantly increasing and therefore a more accurate assessment of alcohol consumption is necessary. In addition to self-report questionnaires, serological biomarkers are useful for assessing alcohol consumption and abstinence, particularly in patients awaiting liver transplantation. The discovery of biomarkers with longer persistence in the body and which can be used to identify alcohol consumption over a longer period of time may be useful in clinical practice [47].

Indirect biomarkers are serological tests that reflect the toxic effect of alcohol on organs and tissues. They are part of the panel of routine laboratory tests and include mean corpuscular volume (MCV), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), and a specific biomarker of hazardous alcohol consumption, carbohydrate-deficient transferrin (CDT).

Direct biomarkers, which persist in the body for a longer period of time and can be tested positive for up to 4 weeks, are metabolites of the non-oxidative pathway of alcohol, are less influenced by the presence of liver disease [24]. Ethylglucuroid (EtG) and ethylsulfate (EtS) can be detected for up to 80 hours in urine. Phosphatidyl-ethanol (PEth), can be dosed after heavy alcohol consumption, having a half-life of 6 days and persisting in the body for up to 3-4 weeks.

Alcohol consumption is based on the presence of common mental states: depression, anxiety and stress. To highlight them, we used the DASS-21 questionnaire. According to a grid, the scores are added up and 5 levels (from normal to extremely severe) of the three states are obtained. The objective of this study was to evaluate with the help of biomarkers, the recent alcohol consumption and the assessment of their condition, in order to refer them for short psychotherapeutic interventions to achieve abstinence.

PATIENTS AND METHOD: We constituted a group of 54 patients with liver cirrhosis determined only by alcohol consumption without other causes. They were assessed for common clinical and paraclinical characteristics and tested for alcohol consumption using the indirect

biomarkers AST, GGT, MCV and CDT, but also an available direct biomarker PEth. The values obtained for PEth were transformed according to the threshold levels reported in the literature into three groups by categories of consumption: values between 0 and 20 ng/l represent the absence of alcohol consumption, between 20 and 200 ng/l represent social, rational consumption, over 200 ng/l is alcohol abuse. His sample of patients was classified into three clusters according to the level of consumption determined by PEth. Patients were asked to consent to answer questions on the DASS-21 questionnaire. According to the obtained score, they were classified as normal, minimal, moderate, severe or extremely severe.

RESULTS AND DISCUSSION: The aim of this study was to assess the level and frequency of alcohol consumption, by confirming with direct biomarkers that detect excessive consumption in patients with alcohol-induced cirrhosis. Our study showed that 63% of the subjects have a moderate consumption and 24% abuse of alcohol despite having a severe condition caused by alcohol. the second aim, the association between alcohol consumption and the intensity of common mental disorders and the detection of patients who need psychological and therapeutic support represented another point of interest, given that these patients have a background of trauma or repeated negative experiences. Biomarkers are part of routine serological determinations (MCV, ALT, AST. GGT) have limited efficiency in detecting heavy alcohol consumption but are even less recommended in moderate consumption. More accurate results can be obtained from the combination of several biomarkers, associations of biomarkers, GGT is interpreted together with CDT. In our study we obtained a weak but significant correlation with PEth and the two markers, although the chosen sample consisted of subjects with advanced liver damage. Carbohydrate-deficient transferrin (CDT) can help identify relapse after a period of abstinence, a condition required prior to listing. [48]. The calculated threshold value for CDT 0.65% is lower than that reported in the literature, but given that the studied patients are cirrhotic it is possible that this indicates excessive alcohol consumption. For GGT the threshold value was 129 IU/l but it was not significant in differentiating excessive consumption from moderate consumption. Thus, with the help of PEth and the threshold values previously defined in the literature, we were able to determine both episodic abuse and chronic abuse, as well as moderate/rational alcohol consumption. The emotional state of the cirrhotic patient that triggers and maintains alcohol consumption despite the presence of liver disease serious of the same cause was determined with the help of the results obtained in the DASS 21 questionnaire. The most frequently associated excessive alcohol consumption with a severe level were stress and

depression. Anxiety was only moderately associated. Evidence of the diagnosis is necessary for the complex therapeutic approach of this pathology.

In the literature, the most common disorder is anxiety with a prevalence of 20-40% [49-50], and alcohol appears to relieve anxiety in the short term. And major depression has the same frequency among alcohol users [51-52], stress, especially post-traumatic stress is associated with alcohol abuse in 15-30% of cases [53]. The association with a common mental disorder with a high degree of severity requires specialized treatment that must be instituted urgently.

CONCLUSIONS: The combination of indirect biomarkers is superior in establishing the diagnosis of alcoholic cause, compared to each marker taken individually. Direct biomarkers are the most sensitive in detecting alcohol consumption and therefore can be used to monitor abstinence in patients awaiting transplantation. Alcohol abuse has as a risk factor a common mental disorder and an initial psychological assessment would have a significant impact on reducing alcohol consumption or even achieving abstinence in these patients. Some of these involve high levels of stress and depression and moderate levels of anxiety, which can be managed with the help of addiction specialists. The development of a diagnostic model based on both marker response and mental disorder assessment questionnaires would be useful to initiate therapy of the cause leading to this type of alcohol consumption.

Study 4. Survival and transplantation at 12 months of patients with alcoholic liver cirrhosis

INTRODUCTION: The prolongation of the waiting time for liver transplantation is due to the insufficient number of donors compared to the demand and need for organs and results in an increasing pre-transplant mortality. Mortality risk factors identified malnutrition severe, with loss of muscle mass and prolonged hospitalization, frequent admissions to intensive care units, but also low serum sodium values and the absence of an obvious cause of liver cirrhosis have all been associated with pretransplantation death [54], decompensation with the onset of ascites, complications of cirrhosis, especially variceal hemorrhage, but also previous emergency surgical interventions such as complicated umbilical hernia [55]. they all increase the risk of death. Alcohol abuse had a lower survival rate and the association with mental disorders further increases the risk of death [56].

PATIENTS AND METHOD: In this study we evaluated the patients from the first batch of 175 and for the survival related to the presence of sarcopenia (considered by the cut-off values of

the psoas muscle index) we took into account the 133 patients (restricted batch). They were followed at 1, 3, 6, and 12 months at the end of the study, for death or transplantation as the final event. Alcohol consumption at time T0 was assessed by the AUDIT-C questionnaire and we used the threshold value of the score to divide the group of patients into moderate drinkers, in which we also included non-drinkers and the second group was of drinkers of exaggerated amounts of alcohol . At the time of first presentation, information was also obtained about the duration of abstinence at that time, based on which four groups were obtained: lifetime abstinence, > 1 year, < 1 year and < 3 months which we considered active.

RESULTS AND DISCUSSIONS: Alcohol-only or mixed etiology had a significantly lower risk of death when these patients were abstinent $p = 0.044$ for the combined cause and $p = 0.043$ for the alcohol-only cause. An increased risk of death at 12 months was in those with nonalcoholic etiology compared to those with mixed etiology 2.67 times higher and 3.3 times higher than alcoholic etiology. This increased risk can be explained by the fact that they mostly had a viral cause and due to the persistence of the etiological factor the disease progresses. The risk of these patients is significantly 3.8 times higher than the risk of former alcohol users ($p = 0.021$).

If we take into account the duration of abstinence declared at baseline, the best survival was in patients who stopped drinking alcohol for more than a year, significantly different from those who never drank alcohol $p = 0.004$, with a median survival estimated 11.73 months, compared to nondrinkers with an estimated average duration of 9.3 months. The differential mortality according to the etiology of cirrhosis shows us once again that those due to alcohol consumption have a better survival than the non-alcoholic/viral ones due to the disappearance of the risk factor, alcohol consumption, compared to the viral ones in which the causative factor is still present,

Regarding survival of sarcopenic patients on the waiting list the difference was significantly better for nonsarcopenic patients $p = 0.005$, with a difference in estimated survival times of 2 months, thus sarcopenia is a significant risk factor for death on the waiting list and represents a complication that requires more attention in the therapeutic management of the cirrhotic patient.

For liver transplantation the estimated waiting time was shorter for patients who consumed alcohol rationally or socially or who did not consume alcohol at all. Their access to transplantation was significantly faster $p = 0.049$.

There were no significant differences in the selection of patients as recipients at 12 months ($p = 0.682$) by length of abstinence from alcohol consumption prior to listing, however those with

lifetime abstinence waited a longer shorter, compared to former users or those who had active use until listing. Nor was the etiology of liver cirrhosis a selection criterion for liver transplant recipients p = 0.907 and the estimated waiting time was relatively equal for all three etiological groups.

Presented malnutrition and sarcopenia were predictors for the access of these patients to liver transplantation after a significantly shorter waiting time interval of 10.2 months, p = 0.04, compared to nonsarcopenic patients after an estimated time interval of 11.4 Monday.

We found no difference in survival between moderate and heavy drinking in patients with cirrhosis, and consumption of 1-2 standard drinks/day does not reduce the risk of death when there is severe liver damage. Regarding survival according to the etiology of the liver disease, there were marginally significant differences, with better survival for patients with disease caused by alcohol consumption or mixed causes, once alcohol consumption is stopped, the determining factor disappears, in cirrhosis related only of alcohol consumption and probably the main factor of mixed cirrhosis. The treatment of choice for liver cirrhosis is liver transplantation and especially for alcoholic cirrhosis with its increasing incidence. The rule of 6 months of abstinence before being listed is also maintained in our center but nevertheless the average waiting time for an organ was relatively equal between patients who had moderate or excessive consumption before listing. Nutritional status with the presence of sarcopenia was a criterion for reducing the waiting time for transplantation.

CONCLUSIONS: Once abstinence from alcohol consumption is established, survival in the following 12 months improves, with equal chances regardless of the type of consumption prior to listing. The greatest decrease in mortality is seen in patients with long-term abstinence > 12 months. The etiology of cirrhosis related to alcohol consumption alone or combined with others has a higher chance of survival compared to non-alcoholic etiology, by stopping the causative agent. Sarcopenia was a predictive factor for liver transplantation and a risk factor for mortality on the waiting list, regardless of the etiology of liver disease.

Conclusions and personal contributions

The data resulting from the studies carried out on patients with liver cirrhosis presented to the clinic at the first hospitalization for evaluation of transplantation, shows, in accordance with the

data in the literature, that a change in the etiology of liver cirrhosis as an indication for transplantation is recorded, from the viral etiology to the alcoholic.

• The sample of patients with liver cirrhosis being evaluated for liver transplantation comprised mostly male subjects 77.71%. Mean age at diagnosis was 52.91 ± 10.29 years, with a median of 54 years

• The most frequent etiology was represented by the combined etiology of alcohol consumption with other causes (48%) and among these other causes, most were secondary to hepatitis virus infections (46.86%). The next most frequent etiology was that related only to alcohol consumption (28.57%) and the other etiologies not related to alcohol consumption (23.43%).

• At the time of admission, based on the clinical interview, 37.7% declared active alcohol consumption in the last 3 months and 20% had recent abstinence of less than a year. The amount of alcohol consumed was above the low-risk level in 59.43% of the entire group of patients and 78% of those with alcoholic cirrhosis declared alcohol consumption in high-risk amounts.

• The frequency of weekly, daily or almost daily alcohol consumption was declared by 57.14% in the entire group of patients and 80% of cirrhotics with alcoholic etiology. The most consumed drinks were wine 44% and distilled drinks 41.71%.

• Giving up alcohol is the first therapeutic line for all alcohol-induced diseases, and liver transplantation is the only curative treatment of decompensated alcoholic liver cirrhosis with the mandatory condition of maintaining abstinence.

• Complex evaluation of these patients is required to be included on the liver transplant waiting list. The use of screening questionnaires yields additional information about alcohol consumption in patients with combined etiology. The multivariate analysis considers as significant for the alcoholic etiology or the alcoholic component in the combined etiology the CAGE and AUDIT-C questionnaires whose score is similar for the two etiologies. Serum GGT levels and MELD-Na score were significantly associated only with the alcoholic etiology of cirrhosis with threshold values above 67 IU/l and above 17.5 points but not with the combined one.

• While ALT was negatively associated with the threshold value above 67 IU/l, being a predictor for the non-alcoholic cause.

• Evaluation for the presence of sarcopenia, especially in patients listed for liver transplantation is essential during the waiting period and for prioritizing them before they are "too sick to be transplanted". The psoas muscle is unaffected by the presence of ascites, a frequent

complication of cirrhosis, and therefore represents the optimal option for determining muscle mass. The psoas muscle index is the optimal option for determining the presence of sarcopenia.

• In the conducted study, the multivariate analysis highlighted male gender ($p = 0.03$) and decompensation with the onset of ascites (small $p = 0.012$; medium $p = 0.011$; large $p = 0.010$) as predictors for sarcopenia.

• Sarcopenia is a complication of liver cirrhosis and its presence does not depend on the etiology of cirrhosis. However, sarcopenia was 2 times more frequent in alcoholic and combined cirrhosis, but without reaching statistical significance.

• Body mass index was significantly associated with the presence of sarcopenia in the group of patients with normal BMI compared to the group of obese patients $p = 0.0054$.

• Anthropometric parameters evaluated in cirrhotic patients, arm circumference, skinfold, weight and body mass index, correlate positively with psoas muscle index values $p < 0.001$, but did not correlate with sarcopenia. These parameters can only suggest a decrease in muscle mass when they have values below the threshold results from the ROC analysis: MAC 25.5; TSF 4.75; G 74.5 and BMI 24.5.

• Prolonged hospitalization ($p = 0.008$) and admission of patients to the intensive care unit (0.021) were independent predictors for the presence of sarcopenia, but in the multivariate analysis they were no longer significantly associated.

• Biomarkers are another useful tool for determining recent alcohol consumption. It is known that the alcohol consumed is underreported in the screening questionnaires, so that the patient can keep his transplant candidacy. Between the indirect biomarkers CDT, GGT and the new direct biomarker PEth we highlighted a positive Spearman correlation with statistical significance $p = 0.002$ CDT and $P = 0.038$ GGT. AST and MCV were not significantly correlated.

• Using the ROC curve we obtained a threshold value for CDT of 0.65%, with good sensitivity 76.9% but low specificity 56.1% and statistical significance $p = 0.038$ for alcohol abuse. The threshold value obtained for GGT was not significant at the threshold value of 129 IU/l with sensitivity 53.8% and specificity 73.2%.

• Patients with alcoholic liver cirrhosis face two health problems: the first is the liver disease itself and the second is the alcohol use disorder underlying a common behavioral disorder. Evaluating patients with excessive alcohol consumption using the DASS 21 questionnaire for depression, anxiety and stress, we obtained association with a severe degree of depression and

stress and a moderate degree of anxiety. That is why the care of these patients must be done in a multidisciplinary team, hepatologists together with psychologists specialized in addictions.

• With prelisting abstinence established and maintained throughout the transplant wait, prolonged abstinence was a good predictor of increased survival through improved liver function.

• There were no significant differences in one-year survival between cirrhotics who had moderate alcohol consumption and those who drank excessively.

• Patients with alcohol-related cirrhosis whose liver function recovered had a significantly improved survival.

• Sarcopenia which was present in 18% of the subjects examined was a negative predictive factor for survival with a difference from nonsarcopenics $p = 0.005$.

• The selection of patients for transplantation did not differ according to consumption or the period of prelisting abstinence or the etiology of cirrhosis, but the presence of sarcopenia was a significant selection criterion $p = 0.04$ associated with the classical criteria.

We believe that the objectives proposed as a research theme stated at the beginning of the paper have been met and we have obtained a series of useful information for medical practice but also for the continuation of scientific research.

Personal contributions

1. Alcohol consumption should be assessed in all patients admitted for listing for liver transplantation. Short specific questionnaires or short versions of complex questionnaires that have proven effective in detecting chronic excessive alcohol consumption in cirrhotic patients can be applied. The score of two questionnaires, CAGE and AUDIT-C were significant as predictors for alcohol etiology of cirrhosis.
2. For a definite diagnosis of alcoholic liver disease and the importance of the alcoholic component in cirrhosis with mixed etiology, it is recommended to combine screening tests and indirect biomarkers, and when direct biomarkers are available, they can be used to detect recent alcohol consumption without had false positive values.
3. We tried to objectively evaluate the common mental disorders depression, anxiety and stress, which are the basis of alcohol consumption and the need to include the patient in a rehabilitation program.

4. Nutritional status is recommended to be assessed in all patients on the waiting list, because malnutrition with sarcopenia is a frequent complication of advanced liver disease, being twice as often associated with alcohol-induced disease and associated with a significantly higher mortality on the waiting list.
5. Survival of patients with alcoholic cirrhosis improves with abstinence, and the chances of being transplanted are equal in all patient groups with maintenance of abstinence.

Future research directions

• Testing patients awaiting transplantation, to maintain abstinence with the help of direct biomarkers, and especially post-transplant patients for alcohol relapse and liver graft compromise.
• Evaluation on the CT or MRI imaging examination performed routinely as part of the pre-transplant balance of the psoas muscle mass with the calculation of the PMI for the classification of patients from the point of view of the presence of sarcopenia and their pre-transplantation, the increase in mortality among sarcopenic patients on the waiting list being documented .
• Screening for alcohol use and common mental disorders using self-report questionnaires is useful for detecting abuse and the depression, anxiety and stress that underlie this behavior with disastrous effects on the body, and the help of a psychologist specializing in addiction is absolutely necessary

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