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BIOCHEMICAL CHANGES IN LIVER CIRRHOSIS

DOCTORAL THESIS ABSTRACT

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Scientific papers

- **Cioarca-Nedelcu R**, Valeriu A, Stoian I. Alcoholic liver disease-from steatosis to cirrhosis-a biochemistry approach. J Med Life. 2021 Sep-Oct; 14(5): 594–599. doi: 10.25122/jml-2021-0081 PMCID: PMC8742892. (capitol 1.2, pag 38-47)
  https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8742892/

- **Cioarca-Nedelcu R**, Kundnani N R, Sharma A, Nistor D, Maghet E, Atanasiu V, Stoian I. Serum biomarkers predictive of cirrhosis in alcoholic liver disease as an alternative to ARFI-SW elastography. Eur Rev Med Pharmacol Sci. 2023 Jun; 27(12):5590-5595. doi: 10.26355/eurrev_202306_32797 PMID: 37401296. **ISI – IF: 3,7.** (capitol 4, pag 52-85)
  https://pubmed.ncbi.nlm.nih.gov/37401296/

- **Cioarca-Nedelcu R**, Atanasiu V, Istratie B E, Nistor D, Proks M, Maghet E, Preda M, Stoian I. Liver cirrhosis in a patient with alcohol dependence and autoimmune hepatitis. Eur Rev Med Pharmacol Sci. 2024 Apr;28(8):3099-3103. doi: 10.26355/eurrev_202404_36025 PMID: 38708468 (capitol 5, pag 86-108)
  https://pubmed.ncbi.nlm.nih.gov/38708468/

Introduction

Liver cirrhosis represents an important public health issue. It is difficult to estimate the prevalence of this pathology in the general population because, most of the time, liver cirrhosis is underdiagnosed. The main causes responsible for the onset of cirrhosis are chronic alcohol consumption, viral infections with hepatitis B, D, and C viruses, and non-alcoholic steatohepatitis [1].

I will further present the reasons that led me to choose this topic for my doctoral research and, at the same time, the socio-economic implications of alcoholic liver disease at a global level. Alcoholic liver disease is a major public health problem, with significant implications for morbidity and mortality. The term "alcoholic liver disease" is the general term used for all liver conditions caused by alcohol consumption. The spectrum of this pathology is broad and can range from asymptomatic hepatomegaly to severe liver failure.

Alcoholic liver disease encompasses a variety of liver injuries, the most representative of which are steatosis, steatohepatitis, fibrosis, and cirrhosis. Steatosis is the first response of the liver to excessive alcohol consumption, characterized by the accumulation of triglycerides in hepatocytes. This can progress to steatohepatitis, a more severe and inflammatory form of liver injury. Alcoholic steatohepatitis can promote the development of liver fibrosis, a process in which extracellular matrix proteins are excessively deposited. The fibrotic response begins with active pericellular fibrosis, which advances to cirrhosis, characterized by excessive and irreversible scarring of the liver parenchyma. As a result, cirrhosis leads to impaired liver function, which can ultimately result in liver failure and portal hypertension [2].

Both early diagnosis and correct staging of liver fibrosis are among the most important procedures in the management of patients with alcoholic liver disease. Although liver biopsy is currently considered the gold standard for staging liver fibrosis, its invasive nature and associated risks have led to the emergence and development of other methods with a superior safety profile.

Traditional imaging methods for detecting and staging liver fibrosis include magnetic resonance imaging (MRI) and computed tomography (CT). However, one of the most

commonly used methods today for detecting and staging liver fibrosis is hepatic elastography, whether or not it is associated with abdominal ultrasound, having the advantages of being non-invasive and very easy to perform.

In addition to elastographic methods for diagnosing and staging liver fibrosis, it is worth mentioning scoring systems that use biochemical markers such as the aspartate aminotransferase to platelet ratio index (APRI) and the fibrosis 4 index (FIB-4).

The main advantages of these biomarkers are their non-invasiveness, wide availability, and affordability. However, to date, APRI and FIB-4 have been primarily developed and used for the diagnosis of advanced fibrosis and cirrhosis in patients with chronic viral hepatitis C. There is currently limited data on the applicability of these scores in patients with alcoholic liver disease.

Contents of the Doctoral Thesis

This doctoral thesis is organized into two sections: a general part, which includes Chapters 1 and 2, and a special part, which comprises Chapters 1, 2, 3, 4, 5, and 6.

General Part

Chapter 1: Describes the extent of the pathology of alcoholic liver disease and the necessity for discovering new non-invasive methods for diagnosing and staging liver fibrosis.

Chapter 2: Covers the definition, epidemiology, clinical and paraclinical presentation, complications, and treatment of liver cirrhosis.

Special Part

Chapter 1: Presents the main biochemical mechanisms involved in the sequence from steatosis to steatohepatitis, fibrosis, and cirrhosis.

Chapter 2: Illustrates the working hypothesis and the general objectives pursued.

Chapter 3: Describes the general research methodology of this doctoral thesis.

Chapter 4: Evaluates the APRI and FIB-4 fibrosis scores as diagnostic and staging methods for liver fibrosis, compared to the diagnostic performance of ARFI-SW elastography. The study results indicate the potential use of both scores for detecting F4 fibrosis in patients with alcoholic liver disease in the study group.

Chapter 5: Presents a case study of a patient initially considered to have alcohol-induced liver damage. After a detailed medical history and additional paraclinical investigations (including the above-mentioned fibrosis scores) and specific immunological tests (ANA, ASMA, antiLKM1), the final diagnosis was autoimmune liver cirrhosis in a patient with chronic alcohol consumption.

Chapter 6: Summarizes the general conclusions derived from this doctoral study, as well as the personal contributions within this thesis.

I. General part

1.Introduction

This doctoral thesis focuses on determining the biochemical changes that occur in liver cirrhosis of alcoholic etiology, as part of the spectrum of alcoholic liver disease.

The main objective of the thesis is to determine a non-invasive method for detecting cirrhosis in patients with chronic alcohol consumption. The method should be inexpensive, based on easily determinable biochemical markers, and integrated into predefined scores in the literature (e.g., APRI and FIB-4). It should have the same diagnostic power as other non-invasive but more expensive methods for evaluating fibrosis (e.g., ARFI point Shear Wave elastography) [4].

Additionally, I aimed to highlight the importance of the differential diagnosis of alcoholic cirrhosis with other existing pathologies, using the medical case presented in Chapter 5 as an example.

2. Liver cirrhosis

Liver cirrhosis (LC) can be defined as the final stage of all chronic liver diseases. It is characterized by extensive fibrosis that leads to the reorganization of liver structure and the formation of regeneration nodules, sometimes accompanied by simultaneous inflammation and hepatocyte necrosis. The cirrhotic progression occurs gradually over many years, sometimes even decades. Regardless of the etiological factor of cirrhosis, the necrosis and inflammation characteristic of chronic hepatitis gradually lead to healing through the formation of collagen bands that disrupt the liver's normal structure.

The main etiological factors of liver cirrhosis are: excessive alcohol consumption; chronic infection with hepatotropic viruses B, C, and D; primary sclerosing cholangitis; primary biliary cirrhosis; secondary biliary cirrhosis due to chronic biliary obstructions; autoimmune hepatitis; drug-induced hepatitis (oxiphenisatin, amiodarone, methotrexate, isoniazid); nutritional disorders (chronic malnutrition, short bowel syndrome, intestinal bypass); hemochromatosis – excessive iron deposition in the liver; Wilson's disease – excessive copper deposition in the liver due to ceruloplasmin deficiency; alpha-1 antitrypsin deficiency; glycogen storage diseases – excessive glycogen deposition in the liver; cardiac cirrhosis (in congestive heart failure); Budd-Chiari syndrome (thrombotic occlusion of the hepatic veins); veno-occlusive disease; idiopathic portal fibrosis; and cryptogenic cirrhosis [1].

From a pathological standpoint, liver cirrhosis is identified by the presence of fibrotic septa that connect central lobular areas either with each other or with portal spaces, thus delineating the regeneration nodules, which are essentially groups of hepatocytes without the central venule. This is the central mechanism through which hepatic microvascularization is distorted, eventually leading to the disruption of the hepatic portal and arterial blood supply [5].

The appearance of regeneration nodules disrupts the hepatic lobule both structurally and functionally because the exchanges in the space of Disse, between the hepatic sinusoids and the neighboring liver parenchyma, are severely compromised. In liver cirrhosis, the hepatic sinusoids lose the fenestrations in the vascular endothelium, and the space of Disse becomes saturated with fibrotic tissue. Ultimately, hepatocytes lose their basic functions, resistance to blood flow through the liver increases, and this culminates in the development of portal hypertension and ascites [6].

Clinical Presentation

In liver cirrhosis, patients can exhibit a wide range of symptoms, from being completely asymptomatic to showing specific signs of liver failure and portal hypertension (edema, ascites, sclero-cutaneous jaundice, upper gastrointestinal bleeding due to the rupture of esophageal-gastric varices). In the early stages, patients may be completely asymptomatic, as mentioned. The earliest symptoms are often asthenia, fatigue, anorexia, and abdominal discomfort associated with a feeling of bloating, both of which can be caused by portal hypertension and ascites.

In prolonged cholestasis (acute alcoholic hepatitis with sclero-cutaneous jaundice, primary biliary cholangitis, or sclerosing cholangitis), pruritus caused by excess bile salts in the skin is a specific symptom. Additionally, dyspnea due to restrictive pulmonary syndrome can occur in patients with large ascites or hydrothorax. Fever may occur during episodes of major cytolysis (acute alcoholic hepatitis, fulminant acute liver failure) and in the context of infectious complications (e.g., spontaneous bacterial peritonitis).

Women may experience menstrual cycle disorders due to hyperestrogenism, which inhibits the hypothalamic-pituitary-ovarian axis responsible for ovulation, leading to amenorrhea.

Biological Changes in Liver Cirrhosis

Biologically, liver cirrhosis presents the following changes:

- Cytolysis Syndrome: Defined by increased transaminases (AST, ALT), usually not very high. Acute cytolysis episodes can occur in acute alcoholic hepatitis or autoimmune liver cirrhosis.
- Cholestatic Syndrome: Characterized by increased total bilirubin with a predominance of direct bilirubin, increased gamma-glutamyl transferase (especially in alcoholic cirrhosis), and alkaline phosphatase (particularly in primary biliary cholangitis and sclerosing cholangitis).
- Inflammatory Syndrome: Characterized by hypergammaglobulinemia: IgG in autoimmune cirrhosis, IgA in alcoholic cirrhosis, and IgM in cirrhosis secondary to sclerosing cholangitis and primary biliary cirrhosis.

- Hepatoprival Syndrome: Due to hepatocellular insufficiency and occurs in advanced cirrhosis.
- Hematological Findings: Mainly feature hypersplenism secondary to splenomegaly caused by portal hypertension.
- Macrocytic Anemia: Common in alcoholic cirrhosis due to associated vitamin B12 deficiency.
- Leukocytosis: Appears in superinfections, the most common example being spontaneous bacterial peritonitis in cirrhotic patients, or in Zieve's syndrome (acute alcoholic hepatitis superimposed on cirrhosis, characterized by jaundice, severe hyperlipidemia, and hemolytic anemia) [8].

Diagnostic Methods

Abdominal Ultrasound is a commonly used investigation for diagnosing liver cirrhosis. In the initial stage of cirrhosis, the liver structure may appear normal, possibly only associated with splenomegaly (anteroposterior spleen diameter ≥ 12 cm). In advanced cirrhosis, ultrasound changes are strongly suggestive of the diagnosis, and can detect/appreciate:

- Free intraperitoneal fluid – ascites – one of the main signs of portal decompensation in cirrhosis;
- Spleen size – in patients with splenomegaly and venous dilations in the hilum, usually accompanied by hypersplenism, biologically manifested by anemia, leukopenia, and thrombocytopenia;
- Liver parenchyma, which may present:
    - Caudate lobe hypertrophy (anteroposterior diameter over 35 mm), especially in alcoholic patients;
    - Gallbladder wall thickening over 3 mm, requiring differential diagnosis with cholecystitis;
    - Regeneration nodules responsible for the heterogeneous character and irregular liver contour;
    - Dilated portal vein with a diameter over 14 mm, lacking respiratory compliance, and sometimes with reversed flow, detected by Doppler ultrasound; dilated splenic vein with a diameter over 8-9 mm; recanalized umbilical vein.

Hepatic Ultrasound is also the main method for screening and detecting hepatocellular carcinoma.

Elastographic Tests are currently of great importance as they can diagnose and stage liver fibrosis. Main Limitation of Impulse Elastography (FibroScan): It cannot be used in patients with ascites or excessive abdominal adipose tissue.

In both cases, the elastographic wave is highly attenuated, leading to significant measurement errors. Attempts to reduce elastographic wave attenuation by inserting a larger ultrasound probe have been unsuccessful. Significant measurement errors have been demonstrated in the inflammation of chronic hepatitis C [9,10].

In contrast to transient elastography, Acoustic Radiation Force Impulse (ARFI) Shear Wave Elastography integrates the necessary software for measurements into conventional ultrasound machines, with the main advantage of being usable without measurement errors in patients with ascites and obesity.

Upper Digestive Endoscopy can reveal signs of portal hypertension such as esophageal and/or gastric varices and portal hypertensive gastropathy. Gastroscopy also allows for hemostatic maneuvers in variceal or non-variceal bleeding [6,11].

Liver Biopsy is an important diagnostic procedure used to evaluate chronic liver diseases, including viral hepatitis and alcoholic liver disease with morphological entities such as simple alcoholic steatosis, alcoholic steatohepatitis, and cirrhosis. It is performed only after assessing the patient's liver function and coagulation status and can be done percutaneously, transjugularly, or laparoscopically [12]. It allows an objective and comparable evaluation of liver damage by assessing:

- The degree of necroinflammatory activity (A);
- The stage of liver fibrosis (F) [13].

Complications of Liver Cirrhosis

Liver cirrhosis (F4 according to the METAVIR score) is classified based on the severity of the pathology into two stages: compensated and decompensated.

The decompensated stage is histopathologically represented by irreversible fibrosis of the liver parenchyma and clinically manifests as: ascites (stage 3); jaundice (stage 4); encephalopathy (stage 4); variceal bleeding (stage 4) [6].

Treatment

In liver cirrhosis, the main treatment objectives are the cessation of alcohol consumption, antiviral treatment with the eradication of hepatotropic viruses, maintaining the compensated stage of cirrhosis, and preventing cirrhosis decompensation and the occurrence of complications.

II. Special part

1. Introduction

Excessive alcohol consumption represents a global health issue. The liver is the organ most affected by chronic alcoholism as it is the primary site of ethanol metabolism. Alcoholic liver disease encompasses a wide range of hepatic lesions, with the most characteristic being steatosis, steatohepatitis, and fibrosis/cirrhosis.

Given the arguments presented above, this doctoral thesis aims to:

- **Deepen the understanding of the mechanisms through which ethanol causes cirrhosis**, mechanisms presented in the review article titled: "Alcoholic liver disease - from steatosis to cirrhosis - biochemical mechanisms";
- **Validate and establish the two scores - APRI and FIB-4 - as new markers for detecting cirrhosis in alcoholic patients**, compared to ARFI-SW elastography in the retrospective study presented in the original article titled: "Serum markers predictive for cirrhosis in alcoholic liver disease - a diagnostic alternative to acoustic radiation force impulse elastography (ARFI-SW)";
- **Highlight the importance of differential diagnosis of alcoholic cirrhosis with other existing pathologies**, exemplified by the case report article: "Hepatic cirrhosis in a patient with autoimmune hepatitis and chronic alcoholism."

Through these objectives, the thesis aims to contribute to the understanding and improvement of the diagnosis and treatment of alcoholic liver cirrhosis, emphasizing the importance of accurate and efficient identification of this severe condition.

2. Working Hypothesis and General Objectives

Hypothesis: The use of APRI and FIB-4 fibrosis scores as substitute tests for ARFI-SW elastography in diagnosing alcoholic cirrhosis.

Objective: To compare the diagnostic performance of the APRI and FIB-4 scores with that of ARFI-SW elastography in detecting patients with F4 (liver cirrhosis) in a cohort of Romanian patients diagnosed with alcoholic liver disease.

Additionally, this study aimed to approximate the most accurate threshold values of the APRI and FIB-4 scores predictive for cirrhosis (using the Youden index and ROC curve analysis), with the goal of reducing the need for ARFI-SW elastography, especially in impoverished regions where this method is not available.

Objectives: a. To record the clinical and paraclinical parameters of the cohort of patients with alcoholic liver disease; b. To stage the degree of hepatic fibrosis using acoustic radiation force impulse (ARFI) elastography of the Shear Wave type; c. To stage the degree of hepatic fibrosis using APRI and FIB-4 scores; d. To compare the diagnostic power of APRI and FIB-4 scores with that of ARFI-SW elastography.

3. General Research Methodology

The study was designed as an analytical, retrospective analysis, including all patients with alcoholic liver disease admitted to the Gastroenterology Department of the Bucharest Emergency Clinical Hospital between January 2019 and December 2020.

For the doctoral thesis, a retrospective analytical study and a clinical case study were conducted. Both studies were in accordance with the Declaration of Helsinki by the World Medical Association and were approved by the ethics committee of the Bucharest Emergency Clinical Hospital.

To carry out the studies, routine demographic information and medical history data (including medical tests and imaging investigations) were collected. The inclusion criteria for the patient cohort in the studies are listed below:

Inclusion Criteria:

- Age > 18 years;
- Presence of ethanol consumption, defined as >20g/day for males and >30g/day for females, assessed according to the AUDIT-C questionnaire recommended by the World Health Organization;
- Clinical and paraclinical diagnosis confirming the presence of alcoholic liver disease.**b.**

Exclusion Criteria: Chronic hepatitis B; Chronic hepatitis B and D; Chronic hepatitis C; Autoimmune hepatitis; Drug-induced hepatitis; Wilson's disease; Hemochromatosis; Heart failure; Significant valvular disease; Hepatocellular carcinoma; Secondary liver conditions; Intra- and extra-hepatic cholangiocarcinoma; Presence of myopathies; Presence of platelet disorders.

4. Predictive Serum Markers for Cirrhosis in Alcoholic Liver Disease - A Diagnostic Alternative to Shear Wave Acoustic Radiation Force Imaging (ARFI-SW)

4.1 Introduction

Hepatic steatosis, steatohepatitis, and fibrosis, along with the final stage of liver damage – cirrhosis – are among the most common histological changes observed in chronic alcohol consumers. Given these types of liver damage, staging hepatic fibrosis is crucial for therapeutic management and prognosis of alcoholic liver disease.

Until now, liver biopsy was considered the gold standard for evaluating hepatic fibrosis; however, due to its invasive nature, alternative methods have been developed. This study examined three of these alternative methods that assess liver stiffness non-invasively. These methods include biochemical markers and scoring systems: the Aspartate Aminotransferase-to-Platelet Ratio Index (APRI) and the Fibrosis-4 (FIB-4) index, as well as Acoustic Radiation Force Impulse Shear Wave (ARFI-SW) elastography.

4.2 Materials and Methods

We conducted a retrospective, analytical study in accordance with ethical principles for medical research involving human subjects as recommended in the Declaration of Helsinki. The study included all patients with alcoholic liver disease admitted to the Gastroenterology Department of the Bucharest Emergency Clinical Hospital from January 2019 to December 2020. Basic demographic information was collected.

Inclusion and exclusion criteria were outlined above. During the period from January 2019 to December 2020, all patients with alcoholic liver disease admitted to the Gastroenterology Department of the Bucharest Emergency Clinical Hospital were included based on the mentioned criteria.

Included subjects were evaluated according to a predetermined protocol, which included medical history, physical examination, and specific paraclinical investigations for the studied pathology.

APRI and FIB-4 scores were calculated using standard formulas:

- APRI: [(AST (U/L) / upper limit of normal) × 100] / Platelet count (10^9/L);

- FIB-4: Age (years) × AST (U/L) / [Platelet count (10^9/L) × (ALT^1/2) (U/L)].

Subsequently, each patient was tested for hepatitis B, C, D, and HIV using serological screening markers.

4.2.4 Assessment of Hepatic Steatosis and Fibrosis

All patients underwent ARFI-SW liver elastography, performed with the patient in deep inspiration, supine position, and the right arm raised above the head for adequate intercostal access for the ultrasound probe.

Excluded patients were those with chronic hepatitis B or C virus infection and/or human immunodeficiency virus infection, acute hepatitis (alanine aminotransferase [ALT] levels ≥10 times the upper limit of normal [ULN]), any type of ongoing infection (e.g., urinary, pulmonary), or any other chronic disease (e.g., heart failure, diabetes, chronic kidney disease).

4.3 Statistical Analysis All data were analyzed using the MedCalc statistical package, version 20.116. Continuous variables were defined by their mean ± standard deviation and/or median (min-max). Diagnostic performance of APRI and FIB-4 scores versus ARFI-SW elastography was analyzed separately based on: sensitivity (Se); specificity (Sp); negative predictive value (NPV); positive predictive value (PPV); and the area under the receiver operating characteristic (ROC) curve.

Optimal threshold values for APRI and FIB-4 scores were determined using the Youden index (Youden Index = Sensitivity (%) + Specificity (%) - 100). The Youden index is a measure of the ROC curve that estimates the effectiveness of a diagnostic marker and simultaneously allows for the selection of an optimal threshold value. A p-value below 0.05 was considered statistically significant.

4.4 Results

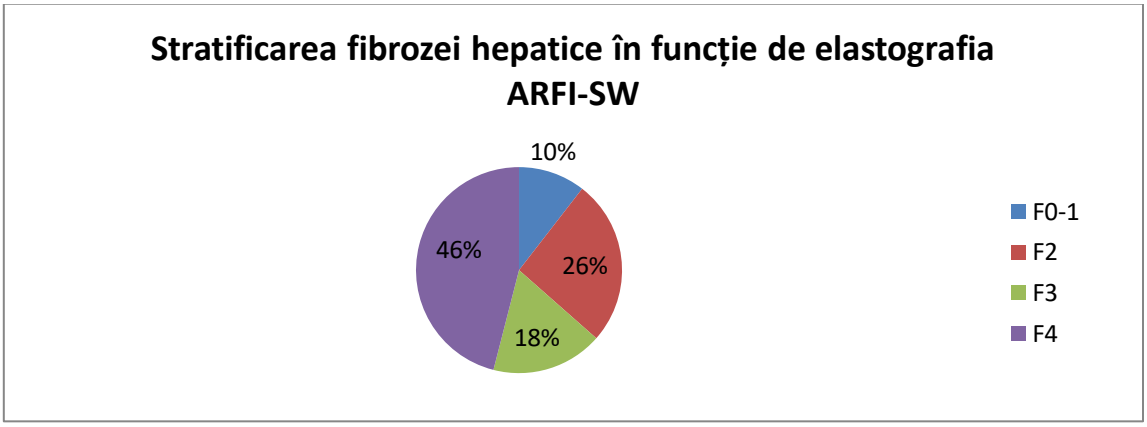
4.4.1 Evaluation of Fibrosis According to ARFI-SW

Elastography Hepatic fibrosis stages according to ARFI-SW elastography (Table V and Figure 4.5) were assessed as follows: F0-1 in 13 patients (10.5%); F2 in 31 patients (26%); F3 in 21 patients (17.5%); F4 in 55 patients (46%).

Table V: Grading fibrosis acording to ARFI-SW

Fibrosis stage	Percentage of patients
F 0-1	10.5%
F 2	26%
F 3	17.5%
F 4	46%

Figure 4.5 Classification of patients depending on the fibrosis stages detected in ARFI-SW elastography



4.4.3 Analysis of the Biological Profile of Study Patients

The biochemical analysis of liver samples indicated that 89.9% of patients exhibited hepatocellular damage characterized by elevated hepatic cytolysis enzymes.

The marker for alcohol consumption—GGT—was elevated in the majority of patients (96.7%), except for those in the decompensated liver cirrhosis stage and those who had been abstinent for the past 3 months.

Cholestasis enzymes were elevated only in a small percentage of patients (10.8%), specifically in cases where acute alcoholic hepatitis was present in addition to the underlying alcoholic liver disease.

Regarding the remaining laboratory tests, these were performed to verify exclusion criteria (criteria related to the presence of liver pathologies whose final evolution could also lead to liver cirrhosis), ensuring accurate differential diagnosis.

Patients were tested for:

- Autoimmune hepatitis (antinuclear antibodies, anti-smooth muscle antibodies, anti-LKM1 and anti-LKM2 antibodies, anti-LC-1 antibodies)
- Hemochromatosis (serum ferritin)
- Wilson's disease (serum ceruloplasmin and 24-hour urinary copper excretion)
- Hepatitis B (HBsAg, anti-HBc total antibodies)
- Hepatitis C (anti-hepatitis C virus antibodies)
- Myopathies (total CK)
- Heart failure (NT-proBNP)

4.4.4 Comparison of Diagnostic Performance of APRI and FIB-4 Scores with ARFI-SW Elastography

Considering the stages of hepatic fibrosis classified by ARFI-SW elastography, we assessed APRI scores (threshold value for F4 > 1.52) and FIB-4 scores (threshold value for F4 > 2.77) as suitable for predicting the presence of liver cirrhosis using the Youden index and ROC curve analysis.

The diagnostic performance of APRI and FIB-4 scores versus ARFI-SW elastography was analyzed separately, focusing on sensitivity (Se), specificity (Sp), negative predictive value (NPV), positive predictive value (PPV), and area under the receiver operating characteristic curve (ROC).

Optimal threshold values for APRI and FIB-4 scores were determined using the Youden index (Youden Index = Sensitivity (%) + Specificity (%) - 100).

The Youden index provides a measure of the ROC curve that estimates the effectiveness of a diagnostic marker and simultaneously allows the selection of an optimal threshold value.

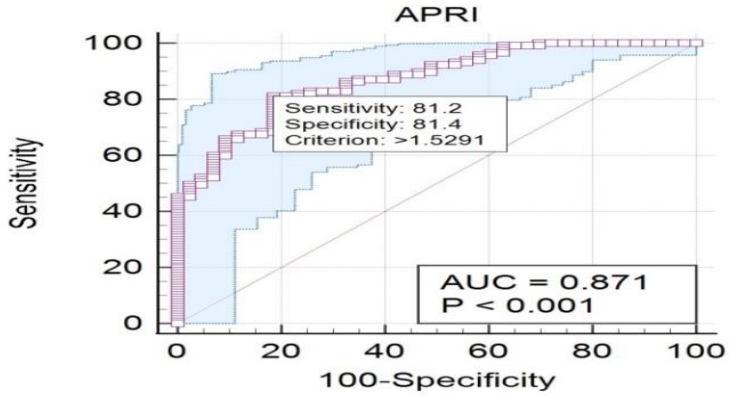
Table VII: Performance indicatores of APRI și FIB-4 in patients with F4 fibrosis

Score	APRI(F4)	FIB-4(F4)
Cut off value	**>1.52**	**>2.77**
Sensibilitaty,%	81.2	83.8
Specificity,%	81.4	74.4
Positive Predictive Value,%	76	73.6
Negative Predictive Value,%	86.1	84.3

The optimal APRI score for patients with F4 was calculated as >1.52 (AUC 0.871, 95% CI 0.809-0.919; P<0.001), providing a sensitivity of 81.2%, specificity of 81.4%, positive predictive value (PPV) of 76%, and negative predictive value (NPV) of 86.1% (Figure 4.6).

A threshold of >1.52 for the APRI score was 81.2% sensitive and 81.4% specific in detecting patients with F4. The PPV for this threshold was as high as 76%, with an NPV of 86.1%.

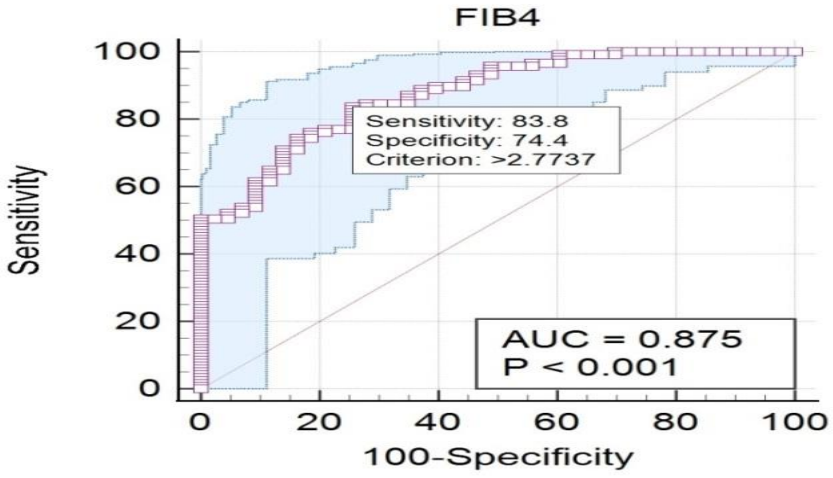
Figure 4.6: Diagnostic performance of the APRI score in predicting patients with F4 compared to ARFI-SW elastography results. ARFI-SW Elastography (Acoustic Radiation Force Impulse - Shear Wave); AUC, area under the ROC curve.



The optimal FIB-4 score for patients with F4 was calculated as >2.77 (AUC 0.875, 95% CI 0.814-0.922; P<0.001), providing a sensitivity of 83.8%, specificity of 74.4%, positive predictive value (PPV) of 73.6%, and negative predictive value (NPV) of 84.3% (Figure 4.7).

A threshold of >2.77 for the FIB-4 score was 83.8% sensitive and 74.4% specific in diagnosing patients with F4. The PPV for this threshold was 73.6%, while the NPV was 84.3%.

Figure 4.7: Diagnostic performance of the FIB-4 score in predicting patients with F4 compared to ARFI-SW elastography results. ARFI-SW Elastography (Acoustic Radiation Force Impulse - Shear Wave); AUC, area under the ROC curve.



4.5 Discussions

The aim of this study, as described, was to compare the diagnostic performance of ARFI-SW elastography with the diagnostic performance of APRI and FIB-4 scores for assessing liver fibrosis F4 (cirrhosis) in patients with alcoholic liver disease.

Our statistical analysis concluded that both APRI and FIB-4 scores are suitable for detecting patients with F4 fibrosis (cirrhosis). The optimal cutoff values for APRI and FIB-4 scores were determined using the Youden index—a summary measure of the ROC curve that estimates the effectiveness of a diagnostic marker and allows the selection of an optimal threshold value, also known as the cutoff point. The AUROC values we detected were 0.871 for the APRI score and 0.875 for the FIB-4 score.

A cutoff of >1.52 for the APRI score demonstrated a sensitivity of 81.2% and a specificity of 81.4% in detecting patients with F4. The PPV for this threshold was 76%, with an NPV of 86.1%.

A cutoff of >2.77 for the FIB-4 score showed a sensitivity of 83.8% and a specificity of 74.4% in diagnosing patients with F4. The PPV for this threshold was 73.6%, while the NPV was 84.3%.

Both APRI and FIB-4 are satisfactory tools for excluding non-cirrhotic patients. It is not unusual for the cutoff values of APRI and FIB-4 scoring systems to vary between studies. Differences in the reference ranges used for AST and ALT levels and variations in patient populations, including the prevalence of cirrhosis, may explain these discrepancies. Several predictive cutoff values for APRI and FIB-4 for cirrhosis have been mentioned in the literature.

Regarding the limitations of our study, the most significant are the retrospective nature of our study and the use of a non-invasive procedure like ARFI-SW elastography for assessing liver fibrosis. Another limitation is the small number of patients with low-grade fibrosis, which made the suggested cutoffs significant only for patients with severe fibrosis/cirrhosis. However, the large number of well-documented cases that met our inclusion criteria allowed us to achieve the study's objectives with accuracy.

4.6 Conclusions

When compared to other similar studies, the main difference is that our study compares the diagnostic performance of APRI and FIB-4 scores with ARFI-SW elastography in patients with alcoholic liver disease, while other studies compare the diagnostic performance of APRI and FIB-4 scores with liver biopsy in patients with chronic viral hepatitis. In this post-pandemic era of economic crisis, the need to simplify the assessment of patients with alcoholic liver disease, especially those in low-income areas where liver elastography and biopsy are not available, is crucial.

Our study concluded that the use of biochemical scores such as APRI and FIB-4 can represent a more cost-effective and easier method for distinguishing non-cirrhotic patients from those with cirrhosis.

5. Liver Cirrhosis in a Patient with Autoimmune Hepatitis and Chronic Alcoholism

5.1 Introduction

In recent decades, numerous studies have identified the coexistence of various liver disorders. Alcoholic liver disease, one of the most frequently diagnosed liver conditions, can overlap with other hepatic pathologies such as chronic viral hepatitis B or C, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, hemochromatosis, or Wilson's disease [15–17].

Autoimmune hepatitis is an inflammatory liver disease characterized by circulating specific autoantibodies, hypergammaglobulinemia, and interface hepatitis observed in histopathological examination. Most patients with autoimmune hepatitis have a genetic predisposition, present HLA DR3 or HLA DR4 surface antigens, and respond favorably to immunosuppressive therapy [18].

5.2 Materials and Methods

The presented case study involves a 45-year-old female patient with a history of chronic alcohol consumption over the last decade (which ceased, according to her statements, 2 years ago), moderate-to-severe untreated asthma, and clinical depression being treated chronically with selective serotonin reuptake inhibitors (Citalopram).

The patient was initially consulted by her general practitioner. Routine blood tests showed signs of cytolysis (transaminases elevated 2-3 times above normal values) and hepatic cholestasis (cholestatic enzymes - GGT and alkaline phosphatase - elevated, along with total and direct bilirubin). As the patient's locality lacked facilities for hepatic evaluation and staging through either elastography or advanced biochemical tests, the general practitioner only calculated the APRI and FIB-4 scores, both indicating F4 (liver cirrhosis).

The patient was then referred to our clinic for further investigation. She presented with symptoms of fatigue, anorexia, scleral jaundice, persistent bloating, and diffuse abdominal pain, both spontaneous and on palpation. The history revealed a previous episode of scleral jaundice 7 months earlier, which resolved spontaneously while she was on oral corticosteroids for an exacerbation of bronchial asthma. Physical examination revealed scleral jaundice, mild ascites, bilateral lower limb edema, and tachycardia.

Viral markers for hepatitis B and C (HbsAg, HbeAg, AntiHbe, AntiHbc total, AntiHCV) were negative. Other tests performed included coagulogram, hemogram, serum biochemical profile, and urinalysis, detailed in Table X. Abdominal ultrasound showed a liver of normal size but with a micronodular structure and irregular contour, mild splenomegaly, slightly dilated portal vein (without thrombosis), and minimal ascites (a thin layer of perihepatic fluid). ARFI-SW elastography also identified fibrosis F4 (cirrhosis).

The patient's symptoms, physical examination findings, blood test results, abdominal ultrasound, and hepatic elastography, combined with her history of chronic alcohol consumption, led to the primary diagnosis of toxic-nutritional liver cirrhosis. However, the rapid resolution of scleral jaundice during corticosteroid treatment raised suspicion of an autoimmune liver disease.

Additional specific tests were requested, including autoantibodies for autoimmune hepatitis, quantitative IgG measurement, serum ferritin, and urinary and serum copper levels, as well as ceruloplasmin to confirm or exclude hemochromatosis and Wilson's disease.

TABle X: Laboratory tests results

Test	Normal range	Results
ALT	10-50 U/L	360 U/L
AST	14-50 U/L	182 U/L
ALP	38-126 U/L	171U/L
Total bilirubin	0,2-1,3 mg/dl	4,6 mg/dl
Proteine totală	6,3-8,2 g/dl	5,9 mg/dl
Albumină	3,5-5 g/dl	2,8 g/dl
Trombocites	150-350X103 /mm3	81.000/mm3
Haemoglobin	12-14 g/dl	8.9 g/dl

Volum corpuscular mediu	79-92fl	103fl
Hematocrite	38-46%	36%
Protrombin time	10-13,2s	17s
INR	0,80-1,15	2,1
Urinary bilirubin	-	Positive
ANA	-	Positivs
ASMA	-	Positive
Anti-LKM 1	-	Negative
Anti-LKM 3	-	Negative
Anti-LC1	-	Negative
Serum IgG	700-1600 mg/dl	2156 mg/dl
Ferritin	30-400ng/ml	68ng/ml
Ceruloplasmin	19-31mg/dl	27mg/dl
Serum copper	63-140μg/dl	Not performed
Urinary copper in 24h	3-50μg/24 de ore	23 μg/24 h
Ag HbS	Non-reactive	Non-reactive
Anti VHC	Non-reactive	Non-reactive

Blood tests excluded the following pathologies as possible causes of liver cirrhosis: viral hepatitis (negative hepatitis B surface antigen and non-reactive anti-Hbc and anti-HCV antibodies), hemochromatosis (normal ferritin levels), Wilson's disease (normal 24-hour urinary copper levels).

However, the blood tests revealed:

- **Macrocytic anemia** (elevated hemoglobin, increased mean corpuscular volume, likely due to folic acid and vitamin B12 deficiency secondary to alcohol consumption);
- **Secondary thrombocytopenia** due to hypersplenism caused by splenomegaly and portal hypertension;
- **Increased prothrombin time** due to clotting factor deficiencies associated with cirrhosis;
- **Cytolysis syndrome** (transaminases elevated 4-5 times above the upper limit of normal);
- **Cholestasis** (elevated GGT, alkaline phosphatase, and total bilirubin);

- **Positive serology** (antinuclear antibodies and anti-smooth muscle antibodies positive) for autoimmune hepatitis type 1.

Liver biopsy could not be performed due to the patient's cirrhotic status, which resulted in thrombocytopenia, elevated prothrombin time, and INR.

The final diagnosis was liver cirrhosis associated with autoimmune hepatitis in a patient with alcohol dependence.

Treatment

In the case of our patient, she was not eligible for immunosuppressive treatment with Azathioprine due to thrombocytopenia resulting from hypersplenism associated with liver cirrhosis.

Budesonide was also not a viable treatment option as it would require extensive hepatic metabolism, which the patient's cirrhotic liver could not support [19].

The only remaining viable treatment option was to initially administer 60 mg/day of Prednisolone, with the dose reduced over 6 weeks to 20 mg/day, and then to maintain a dose of 10 mg/day starting from week 10.

At 6 months of treatment, the patient is stable, without jaundice, ascites, or leg edema.

5.4 Discussion

This case presentation highlights the challenges faced by our medical team in diagnosing autoimmune liver cirrhosis in a patient with a history of alcohol dependence. Both alcoholic liver disease and liver injury induced by medications such as selective serotonin reuptake inhibitors could have been alternative diagnoses. Fortunately, detailed anamnesis and specific blood tests revealed the main diagnostic clues for this case.

Initially, the patient reported that alcohol consumption ceased 18 months before the first episode of jaundice. During this time, two blood tests were performed, both indicating only macrocytic anemia, without cytolysis or hepatic cholestasis.

Subsequently, during oral corticosteroid therapy used to treat one of the patient's asthma exacerbations (which occurred just 2 weeks after the first episode of jaundice), the scleral jaundice completely resolved. Liver enzyme and cholestasis levels returned to normal reference ranges immediately after the first week of corticosteroid treatment.

Ultimately, the most common causes of liver cirrhosis—alcohol consumption, chronic viral infections, and drug-induced liver injury—were excluded, after which the patient was tested for hemochromatosis, Wilson's disease, and autoimmune hepatitis.

Our patient tested positive for ANA and ASMA autoantibodies, leading to the final diagnosis of liver cirrhosis associated with autoimmune hepatitis type 1 in a patient with alcohol dependence. Unfortunately, the patient was not eligible for treatment with budesonide and/or azathioprine due to her cirrhotic status and low platelet count. The final medical decision was to treat her initially with 60 mg of prednisolone, reducing the dose from 60 to 20 mg over 4 weeks. After the first month of corticosteroid therapy, the patient's overall symptoms improved (no ascites, jaundice, or leg edema), as well as laboratory results (normal liver enzymes and absence of cholestasis).

6. Conclusions and Personal Contributions

This doctoral thesis focuses on identifying biochemical changes associated with alcoholic liver cirrhosis as part of the broader spectrum of alcoholic liver disease. The primary objective of the thesis is to develop a non-invasive method for detecting cirrhosis in patients with chronic alcohol consumption. This method should be financially accessible, based on easily measurable biochemical markers, and integrated into standardized scoring systems in the literature (e.g., APRI and FIB-4), with diagnostic power comparable to other non-invasive but more costly methods used for assessing fibrosis (such as ARFI-SW elastography).

The main conclusion of the study was the validation of APRI and FIB-4 scores as reliable tools for diagnosing cirrhotic patients, thereby reducing the need for ARFI-SW elastography, especially in less developed regions where such technology may not be available.

Regarding the importance of differential diagnosis of alcoholic cirrhosis versus other liver pathologies, as exemplified by the case report: "Liver Cirrhosis in a Patient with Autoimmune Hepatitis and Chronic Alcoholism," the final conclusion was as follows: Even though alcoholic liver disease and non-alcoholic fatty liver disease are among the most common causes of liver disorders today, medical personnel should remain vigilant for less common liver pathologies to avoid misdiagnosis or delayed diagnosis and to ensure optimal treatment.

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