VICTOR BABEȘ UNIVERSITY OF MEDICINE
AND PHARMACY FROM TIMIȘOARA
FACULTY OF MEDICINE
DEPARTMENT VII - INTERNAL MEDICINE II

FONCEA A. CAMELIA-GIANINA

PhD THESIS

ALCOHOLIC LIVER DISEASE- A COMPLEX AND CONTROVERSIAL PATHOLOGY

– A B S T R A C T –

Scientific Coordinator
PROF. SPOREA IOAN, MD PhD

T i m i ș o a r a
2 0 2 3

ABSTRACT

GENERAL PART

As a major cause of mortality and morbidity, chronic liver disease (CHD) is a serious global public health problem. The most prevalent causes are now non-alcoholic fatty liver disease (NAFLD), and alcoholic liver disease (ALD), hepatitis B virus (HBV), hepatitis C virus (HCV).

The development of alcoholic liver disease (ALD) and its impact on both individuals and communities are increasing, making it a serious worldwide health concern. The motivation behind research in ALD is driven by a multitude of factors, including the urgent need for a deeper understanding of the disease's mechanisms, the quest for improved diagnostic and treatment approaches, and the desire to alleviate the burden it imposes on society.

An estimated 3.3 million people die each year from drinking too much alcohol. One of the most serious disorders brought on by persistent alcohol use is alcohol-induced liver damage. ALD encompasses a spectrum of disorders, ranging from early fatty liver (liver steatosis and inflammation) to severe alcoholic hepatitis and cirrhosis.

The progression of inflammatory responses to liver damage results in chronic inflammation, which actively promotes the development of liver fibrosis and promotes the onset of cirrhosis and hepatocellular carcinoma (HCC). Alcohol is responsible for almost 50% of all liver cirrhosis cases in Europe. Since liver fibrosis represents a turning point in the development of chronic liver disease, it is one of the most significant prognostic factors.

Liver biopsy is the gold standard for diagnosing liver fibrosis in individuals with ALD. But is an invasive technique with possible complications and errors. Consequently, several noninvasive diagnostics have been developed and are increasingly being used in clinical settings. The two main non-invasive techniques for diagnosing liver fibrosis and liver steatosis are biological markers, which are composed up of various blood tests that are processed using different algorithms, and ultrasound-based elastography methods, which allow for the physical characteristics of the hepatic tissue to be assessed. Non-invasive ultrasound-based liver elastography techniques for the assessment of liver stiffness, such as Strain and Shearwave elastography have become widely available.

Our focus in liver hepatopathies is performed by Shearwave methods (SWE). One of the first and most validated elastographic methods was Vibration-Controlled Transient Elastography (VCTE), followed by other techniques such as point shear wave elastography (pSWE) and two-dimensional shear wave elastography (2D-SWE). Another key-point in

early diagnosis for ALD are serum biomarkers, patented and non-patented such as APRI, FIB-4, AST/ALT, Age-to-platelet-index.

Many patients, unfortunately, present to the doctor in an advanced or decompensation stage, since ALD is still an underestimated liver disease. Alcoholic hepatitis is one of the most severe forms of ALD and can develop on top of any other liver condition. Since there is a 50% chance of death within 30 days from alcoholic hepatitis, early identification is essential for effective treatment.

Implementing appropriate treatment options, such as medication-assisted treatment, supportive care, and alcohol cessation, is feasible by early and accurate diagnosis. In the absence of confounding factors, recent studies rule out liver biopsy as a diagnostic tool for an early diagnosis. In cases of alcoholic hepatitis, the diagnosis and prognosis evaluation are tightly related. A variety of scoring systems are used to assess the severity of the disease and predict the short-term mortality, including the Glasgow Alcoholic Hepatitis Score (GAHS), the Model for End-Stage Liver Disease (MELD), Maddrey Discriminant Function (MDF) and Lille score.

Considering the main objectives of the published studies, the thesis was divided in two major chapters: Non-invasive screening and diagnosis of alcoholic liver disease and Alcoholic hepatitis: prognostic factors.

SPECIAL PART

The primary focus of this thesis focused on individuals with Alcohol Use Disorder (AUD) in connection to their liver health, with a particular emphasis on the critical roles of liver steatosis and liver fibrosis as key prognostic indicators in liver-related diseases. Within this patient cohort, the study used screening methods including as imaging, elastography, and serum marker assays. Novel elastography techniques are being studied to improve the accuracy and utility of diagnosing liver fibrosis.

Furthermore, a specialized subset of patients with severe liver diseases, such as alcoholic liver cirrhosis and alcoholic hepatitis, were evaluated. A study was conducted as part of this evaluation to identify prognostic factors and markers for predicting responses to early therapy. The study focused on two specific diseases that are characterized by advanced liver damage and increased death rates. The major goal was to examine and identify predictive markers that could allow for earlier intervention and improve outcomes for patients diagnosed with alcoholic hepatitis.

Taking these major components into consideration, the thesis part pertaining to these parts was divided into two distinctive chapters: "Screening and Diagnosis of Liver Stiffness and Steatosis" and "Alcoholic Hepatitis."

1. GENERAL OBJECTIVES

1) To determine the importance of screening and early diagnosis in patients with ALD and to establish a screening/diagnosis algorithm.
2) To evaluate the accuracy and reliability of two new elastographiy techniques, such as Point Shear Wave Elastography (pSWE) and two-dimensional Shear Wave Elastography (2D-SWE), for the diagnosis and staging pf alcoholic liver disease using vibration controlled transient elastography (VCTE) as reference method.
3) To determine the optimal cutoff value and thresholds for elastography measurements in the diagnosis and management of alcoholic liver disease.
4) Non-invasive assessment of liver fibrosis and steatosis severity in AUD patients using VCTE with Controlled Attenuation Parameter (CAP) and serum markers.
5) To compare the diagnostic performance of elastography with other noninvasive methods, such as serum biomarkers and imaging techniques.
6) To assess the clinical utility of elastography in predicting disease progression and outcomes in patients with alcoholic liver disease.
7) To identify potential cofounding factors that may affect the accuracy of elastography measurements in patients with alcoholic liver disease, such as inflammation.
8) To evaluate the severity and prognosis of alcoholic hepatitis using established scoring systems, such as the Maddrey Discriminant Function (MDF), the Model for End-Stage Liver Disease (MELD), and the Glasgow Alcoholic Hepatitis Score (GAHS), ABIC scores.
9) Comparison of Lille score at 4 days vs. 7 days for predicting response to corticosteroids and 28-day mortality in patients with severe alcoholic hepatitis (SAH).
10) To identify gaps in the current knowledge and understanding of alcoholic liver disease, and to propose future research directions and priorities.

2. MATERIAL AND METHODS

2.1. SUBJECTS

Prior to participation in the current study's component studies, all subjects provided written consent to conduct elastography measurements as well as clinical, ultrasonographic, and biological examinations. This study was carried out in compliance with the most recent version of the World Medical Association Declaration of Helsinki and was authorized by our university's local research ethics committee and review board (Nr. 27/28.09.2018).

Common inclusion criteria for all subjects in the first two studies including liver elastography were age >18, the ability to sign informed consent, known medical history of liver disease: presence or absence of liver fibrosis, by elastographic measurements . Lack of informed consent, ascites, aminotransferases greater than 3xULN, symptoms of biliary obstruction or liver congestion, and focal liver lesions seen during an ultrasound examination were all exclusion criteria.

For the third study, which included patients with advanced chronic liver disease and alcoholic hepatitis, we utilized the following exclusion criteria to form the study cohort: (1) Corticosteroid contraindications: uncontrolled acute infections, active gastric ulcers, neoplasia, hepatorenal syndrome; (2) chronic underlying liver disease (active hepatitis B or C, probable autoimmune liver disease, or drug-induced liver disease). All individuals included were previously diagnosed with alcoholic liver cirrhosis following well-established criteria.

In the present research, we included a total number of 390 subjects, aged between 19-86 years old (median 54.8 $\pm$ 12.8), out of which 86 (22%) were female and 304 (78%) males. The study was conducted in Department of Gastroenterology and Hepatology, County Emergency Clinical Hospital „Pius Brînzeu", Timișoara, Romania between October 2018 and October 2021 and included patients with alcohol use disorder and alcoholic liver disease in different phases of evolution.

2.2. ULTRASOUND EVALUATION

Abdominal ultrasonography was performed on all patients in the first study using a Samsung Medison RS85 and a CA3-10A convex probe. We first performed standard abdominal ultrasound on all patients to describe the liver's aspect, structure, and surface, as well as specific signs that could lead to a diagnosis or affect liver stiffness measurement, such as biliary obstruction, focal liver lesions, portal vein thrombosis, heart failure, and, most importantly, the presence of ascites.

2.3. ELASTOGRAPHIC EVALUATION

2.3.1. Vibration-Controlled Transient Elastography (VCTE) and Controlled Attenuation Parameter (CAP)

According to the most recent recommendations, the FibroScan Compact 530 (Echosens, Paris, France) was used to determine the degree of liver fibrosis and steatosis. The Standard M or XL probes were selected using the device's built-in automatic probe selection tool software. We used VCTE with CAP as a reference method for the studies in our research, and cut-off values for the diagnosis of different degrees of steatosis and fibrosis varied from one study to the next and were chosen based on pathology and patient characteristics.

2.3.2. Point and 2D- Shearwave Elastography (p-SWE and 2D-SWE) on Samsung Medison RS85

On the Samsung Medison RS85, pSWE of the liver (S-Shearwave) was performed by first using B-Mode imaging to identify the region of interest using the CA3-10A convex probe and followed by 2D-SWE.The S-Shearwave Profile also included the Reliability Measurement Index (RMI) and Variation Range (VR). While an RMI of 1.0 indicates no error, one of the other values, 0.0, indicates significant error. Measurements with RMIs less than 0.4 were eliminated to increase reliability. For both methods, Reliable LS measurements were defined as the median value of 10 measurements, with a RMI≥0.5 and IQR/M≤30%.

2.4. BIOLOGICAL DATA

The following biological scores have been determined for the diagnosis of advanced fibrosis: the aspartate transaminase (AST)-platelet-ratio index (APRI), the Age-platelet index, the Fibrosis-4 index (FIB4), the AST/ALT ratio (AAR), and literature-based cut-off values for advanced fibrosis: APRI≥1.0, Age-platelet index ≥6.0, FIB4≥3.25, AST:ALT ratio >1.0.

To determine the best predictor of early mortality in severe alcoholic hepatitis, the following prognostic scores were generated, and literature-based cut-off values were utilized as markers of severe disease: : ABIC > 9, GAHS ≥ 9, MELD ≥ 21, Lille model score at 4 and 7 days LM4/7 ≥ 0.45.

2.5. STATISTICAL ANALYSIS

Cut-off values for various stages of liver fibrosis were calculated using areas under receiver operating characteristic (AUROC) curves. We employed the Pearson coefficient of precision and accuracy contained in the Lin's Concordance Correlation Coefficient (CCC)

and the Bland- Altman plot analysis to compare the two novel approaches (p-SWE and 2D-SWE) with VCTE. The Chi - square (x2) test (with Yates correction for continuity) was used to compare proportions as percentages in the evaluation of the best predictor score for death prediction in patients with alcoholic hepatitis. Then, for each predictive test, 95% confidence intervals were generated, and p 0.05 was considered significant for each statistical test. For mortality predictors, AUROC curves were generated, and z-scores were calculated for comparing ROC curves.

3. RESULTS

3.1. SCREENING AND DIAGNOSIS OF LIVER FIBROSIS AND LIVER STEATOSIS IN PATIENTS WITH AUD BY NON-INVASIVE METHODS

We conducted a prospective monocentric study including patients with AUDIT-C positive test in whom liver stiffness was assessed by VCTE and serum markers were available. The study included 172 subjects, mean age 56.5±10.4, 156/172 (90.7%) patients were male. All included patients had positive AUDIT-C test, reliable liver stiffness measurements by VCTE and CAP, and no previously known liver disease.

Regarding liver fibrosis, a majority of included subjects, 95/172 (55%) had F1 (mild fibrosis) or no fibrosis, while a significant percentage of included subjects, 30/172 (17.5%), had liver cirrhosis. In contrast to the percentage of participants with liver fibrosis, a significant proportion of 90/172 (52.3%) had severe liver steatosis.

3.1.1. Role of AUDIT-C score and non-invasive fibrosis serum markers for the diagnosis of ALD in comparison with VCTE

The biological scores AST/ALT, APRI, FIB-4, and Age-platelet index were acquired from 106/172 (61.6%) participants.

Using Spearman's correlation, statistically significant correlations were evidenced between LS by VCTE and AUDIT-C values ($r=0.46$, $p<0.0001$), APRI ($r=0.42$, $p=0.001$), FIB-4 ($r=0.31$, $p=0.0012$) and the age-platelet index ($r=0.44$, $p=0.008$).

To identify advanced fibrosis, the following cut-off values were used: APRI 1.0, FIB4 3.25, Age-platelet index 6.0, and AST: ALT ratio > 1.0. The AST/ALT ratio, 55.7% (APRI score), 67% (FIB-4 score), and 58.5% (Age-platelets index) were used to identify participants with advanced fibrosis in 45.3% (48/106) of the instances.

In univariate linear regression analysis, the following independent predictors for the presence of advanced fibrosis were identified: **AUDIT-C** ($p=0.001$), **FIB-4** ($p=0.01$), and **age-**

platelet index (p=0.03). In multivariate linear regression analysis and Akaike information criteria (AIC) to select the best model we found that the model including AUDIT-C (p<0.001) and age-platelet index (p=0.04) was the best model associated with advanced fibrosis.

3.1.2. Performance of non-invasive fibrosis scores and AUDIT-C for predicting advanced fibrosis using VCTE as a reference method

Based on AUROC comparison, Age-platelet index (AUC 0.82) performed significantly better for predicting advanced fibrosis than AST/ALT (AUC 0.55) and APRI (AUC 0.58) (p=0.0001 and p=0.0014, respectively) with no differences when compared to AUDIT-C (AUC 0.74) and FIB-4 (AUC 0.77) (p=0.21 and p=0.35, respectively).

AUDIT-C performed better than AST/ALT (p=0.01), but no differences were found when compared to APRI (p=0.08), and FIB-4 (p=0.75). and age-platelet index (p-0.21), respectively.

The proportion of subjects correctly classified as having advanced fibrosis was significantly higher for FIB-4 than for AST/ALT ratio (p=0.002), while no differences were found between FIB-4 and APRI score (p=0.119) or Age-platelet index (p=0.252), or between APRI and AST/ALT ratio (p=0.072), or between APRI and AST/ALT ratio (p=0.166).

3.1.3. Inflammation-adapted liver stiffness values

When inflammation-adapted liver stiffness data were applied to the group of F0-F2 patients, 5/82 were classified as F3, and 77/82 remained F0-F2. After the AST correction, 9/14 patients with F3 developed F4. In the F4 subgroup, 4/16 patients had F3, and 1/16 had F0-F2, while 11/16 remained F4.

3.2. PERFORMANCE OF NEW ELASTOGRAPHIC TECHNIQUES AS NON-INVASIVE TECHNIQUES INTEGRATED IN THE SAME ULTRASOUND MACHINE FOR PREDICTING LIVER FIBROSIS IN ALD

New elastographic techniques have proved to be accurate in the diagnosis of liver fibrosis in patients with ALD. The study methods were pSWE and 2D-SWE integrated in the same ultrasound machine (Samsung Medison RS85) and VCTE was taken as a reference method. The final study included 101 patients in whom all measurement techniques had reliable data, including VCTE in 98.2% (113/115) patients, pSWE in 93.9% (108/115), and 2D-SWE in 92.1% (106/115) patients. Almost 16% of patients had ALD.

Using the TE cut-offs suggested by the Tsochatzis meta-analysis the classification of liver fibrosis showed: F0-1 (no or mild fibrosis)- 67/101 patients (66.3%), F2-3 (significant or advanced fibrosis) – 16/101 patients (15.8%), and F4 (liver cirrhosis)- 18/101 patients (17.8%).

3.2.1. Performance of new p-SWE and 2D-SWE technique for liver stiffness assessment

Using VCTE as reference method, we calculated the cut-off values for diagnosing significant fibrosis (F≥2) and cirrhosis (F=4). Best cut-off values for pSWE F2 >5.9 kPa, for F4 >8 kPa and for 2D-SWE F2>6.1 kPa and F4 >7.6 kPa . The mean value obtained by pSWE and 2D-SWE were similar: 7.24±5.88 kPa *vs.* 7.26±5.04 kPa, p=0.96.

3.2.2. Correlation between liver stiffness values obtained by VCTE and p-SWE and 2D-SWE

Strong positive correlations between VCTE measurements and 2D-SWE (r=0.85) and pSWE (r=0.88) and between pSWE and 2D-SWE (r=0.90) were found.

The mean difference between TE and p-SWE was 0.9±0.38 according to the Bland-Altman test. The maximum and lower limits of agreement (LOA) with 95% confidence were 8.5 and -6.7 kPa, respectively. TE and 2D-SWE had a mean difference of 0.9±0.4. The 95% upper and lower LOA were 9.7 and -7.9 kPa, respectively , with a mean difference of 0.008±2.3 between p-SWE and 2D-SWE. The upper and lower 95% LOA values were 4.9 and -4.9 kPa, respectively.

3.3. ALCOHOLIC HEPATITIS AND ALCOHOLIC LIVER CIRRHOSIS. EVALUATION OF SEVERITY SCORES FOR PREDICTING THE PROGNOSIS AND TREATMENT RESPONSE

3.3.1. Introduction

We analyzed 103 patients diagnosed with alcoholic liver cirrhosis and alcoholic hepatitis superimposed, out of which 74 (72%) had severe AH with MDF>32 and were included in the final analysis and only 55 (74%) received corticosteroids as a treatment for the main contraindication being severe active infection. In the analyzed subgroup of patients receiving corticosteroid therapy, 18/55 (32%) had minor infections at presentation and corticosteroids were started immediately after antibiotherapy or in combination with antibiotics, while 4/55 (7.3%) had infections after corticosteroid therapy was started. The majority of patients in the Child Pugh C class had previously diagnosed alcoholic liver cirrhosis. In terms of severity scores, 18/55 corticosteroid patients had ABIC>9, 31 patients had GAHS 9, and 48 patients had MELD21.

3.3.2. Comparison between Lille score at 4 days and Lille score at 7 days

The correlation between LM4 and LM7 was 0.94 with a p-value of 0.0001 and an R2 of 0.88, indicating that 88% of LM4 values agreed with those of LM7.

The Bland-Altman test found a mean difference of 0.04 between LM4 and LM7. The 95% upper and lower LOA were 0.25 and -0.16 respectively. In our study group, the percentage of patients who had a responder Lille score value at 4 days versus 7 days was the same (27% vs 36%, p=0.31). 5/55 (9%) of LM4 non-responders turned out to be LM7 responders.

3.3.3. Performance of LM4 and LM7 in predicting 28 days mortality

Nonresponders had a higher 28-day mortality rate (LM4 13.3% and LM7 31%, p = 0.22) than responders (LM4 30% and LM7 15%, p = 0.29), using LM4 and LM7 with a cut-off>0.45. When compared to LM7, LM4 properly identified 90.3% of patients as responders or nonresponders. The Kaplan-Meier survival analysis showed that the mean overall survival was 24.3 days. For responders, the mean survival was 25.1 days and for nonresponders 18 days. Overall survival was 24.3 days on average. Responders had a considerably longer survival time than nonresponders (p = 0.01), with a mean survival of 25.1 days for respondents and 16.3 days for nonresponders. According to the LM4 criterion, responders had a considerably greater 28-day survival rate (85.0% vs. 61%, p = 0.04) than nonresponders.

3.3.4. Predictors for Mortality and Comparison of Different Predicting Scores for Mortality

The MELD >21 score had the best performance to predict mortality with AUC 0.8, followed by Maddrey with AUC of 0.74, and LM7 and LM4 with AUCs of 0.68 and 0.67, respectively. The ABIC and GAHS scores displayed poorer performance, with AUCs of 0.64 (p 0.0001). MELD score, LM4, and LM7 were combined to provide the best model for predicting 28-day mortality, with an accuracy of 0.90 for both combinations. The accuracy of MELD + MADDREY was 0.86, while MELD + MADDREY + LM4/7 was 0.87.

In univariate analysis, the MELD, MADDREY, and LM4 scores were associated to 28-day mortality. LM4> 0.45 increases the probability of death by 7.2 times.

4. DISCUSSIONS

4.1. SCREENING AND EARLY DIAGNOSIS IN ALCOHOLIC LIVER DISEASE

Alcoholic liver disease (ALD) is a prevalent but often underestimated condition, responsible for millions of deaths worldwide. It ranks among the top indications for liver transplantation in Europe and the United States. Given the rising mortality from alcohol-related liver diseases, there is a pressing need for strategies to screen and diagnose ALD early.

Liver fibrosis is a key predictor of disease severity, making early fibrosis diagnosis a primary concern. Liver biopsy, the gold standard for liver disease diagnosis, is invasive and not recommended. Non-invasive methods have emerged, including biological scores derived from serum markers and imaging-based approaches, particularly liver elastography.

Non-invasive tests like indirect serum markers are cost-effective and reproducible but may produce false positives. To address these issues, screening pathways have been established to facilitate early liver disease diagnosis in at-risk populations.

One study in this thesis assessed the need for ALD screening and early diagnosis. Two critical findings emerged: FIB-4 and Age-platelet index performed exceptionally well in predicting advanced liver fibrosis. The presence of advanced fibrosis was independently correlated with FIB-4 ($p=0.01$) and age-platelet index ($p=0.03$). FIB-4 had an AUROC of 0.85 and 91% specificity for the prediction of advanced liver fibrosis in recent research combining direct and indirect markers.

Moreover, elastography-based techniques have shown promise for non-invasive fibrosis evaluation, with Shear Wave Elastography recommended as a preferred method.

VCTE is a predictive tool for liver-related events in early alcoholic liver disease and provides great diagnostic accuracy for advanced fibrosis, with AUROCs > 0.90. VCTE is one of the most used methods in studies for the diagnosis of liver fibrosis, showing good sensitivity and sensibility 87% (95%CI 0.64-0.96) and 82% (95% CI 0.67-0.91), respectively for the diagnosis of advanced liver fibrosis with a cut off value between 11-12.5 kPa.

Vibration-Controlled Transient Elastography (VCTE) emerged as a predictive tool for early alcoholic liver disease, providing excellent diagnostic accuracy. Additionally, VCTE's sensitivity and specificity for diagnosing advanced liver fibrosis were noteworthy.

In this study, a significant proportion of patients were diagnosed with liver cirrhosis (17.5%) and severe steatosis (52.3%). For ALD patients, inflammation levels, specifically AST, played a pivotal role in evaluating hepatic stiffness.

To optimize diagnostic accuracy, inflammation-adapted liver stiffness values were utilized. In a study published by Mueller et al., the AUROC for cirrhosis assessment by VCTE increased from 0.921 to 0.946 when these patients with AST > 100 U/L were excluded, and the specificity increased noticeably from 80% to 90% with a sensitivity of 96%. In our study, we applied inflammation-adapted liver stiffness cut-off formula. Out of 14.3% (16/112) subjects with F4, 11/16 remained F4, 4/16 had F3 and 1/16 had F0-F2. After inflammation-adapted liver stiffness values were calculated based on AST values, the proportion of F4 was reduced from 14% to 10%.

Another study, including 101 patients, evaluated alternative elastography methods, including pSWE and 2D-SWE, which demonstrated several advantages. These methods provided relevant cut-off values for the diagnosis of liver cirrhosis.

Strong positive correlations between VCTE measurements and 2D-SWE (r=0.85) and pSWE (r=0.88) and between pSWE and 2D-SWE (r=0.90) were found. What is particularly noteworthy is that there were no statistically significant differences among these correlations, as indicated by a p-value of *0.37,* demonstrating their utility and comparability.

In a multicenter study which included 570 patients the AUROCs of S-SWE for the diagnoses of ≥F2, ≥F3 and F4 were 0.842, 0.844 and 0.850, respectively and the optimal cut-off values for liver stiffness measurements on S-SWE were >7.0 kPa and >9.7 kPa for ≥F3 and F4, respectively. In our study the best cut-off values obtained were: for pSWE F2 >5.9 kPa, for F4 >8 kPa and for 2D-SWE F2>6.1 kPa and F4 >7.6 kPa .

Another study, the first to describe p-SWE and 2D-SWE performed with a Samsung RS85A, evaluated the accuracy and interobserver reproducibility of (SWE) in staging liver fibrosis, using VCTE as a reference method and the final analysis demonstrated good performance in the diagnosis accuracy of clinically significant (F2) liver fibrosis and liver cirrhosis, with AUROC values of 0.94 and 0.89, respectively.

The study used the Bland-Altman test to evaluate the agreement between different methods for measuring liver stiffness. The results showed that VCTE vs. p-SWE had a mean difference of 0.9 kPa and vs. 2D-SWE, the mean difference was also 0.9 kPa. In contrast, when comparing p-SWE with 2D-SWE, the mean difference was much smaller at 0.008 kPa, but the LOA ranged between 4.9 kPa and -4.9 kPa. These results illustrate the agreement and potential variability among these methods in assessing liver stiffness.

The availability of multiple elastography methods in a single ultrasound machine enhances their practicality and reliability.

However, both studies faced limitations, including the absence of liver biopsy data and small sample sizes. Nonetheless, the research underscores the importance of non-invasive methods for diagnosing ALD and lays the groundwork for a proposed algorithm to enhance early diagnosis and patient management.

4.2. ALCOHOLIC HEPATITIS AND ALCOHOLIC LIVER CIRRHOSIS. EVALUATION OF SEVERITY SCORES FOR PREDICTING THE PROGNOSIS AND TREATMENT RESPONSE

Another to be asked question and concern was related to patients with advanced decompensated and complicated alcoholic liver disease, many of them diagnosed directly with complications. And one of these complications is alcoholic hepatitis (AH) superimposed on liver cirrhosis. Severe alcoholic hepatitis (sAH) is defined by a Maddrey Discriminant Function (MDF) > 32 points and is associated with high mortality (over 50%), morbidity, and infection risk. Corticosteroids, the main and most used therapy for severe AH (sAH), resulted in a 14% reduction in one-month mortality. Current guidelines, such as European Association of Liver Study, recommend using corticosteroid treatment for sAH in the absence of contraindications and response to corticosteroids is assessed by Lille score at 7 days.

Our study, aimed to evaluate early response in treatment by calculating Lille score at 4 days and the value of prognostic scores.

The study included a total of 103 patients, 74 with sAH and only 55 suitable for corticosteroid treatment. In our group, 18/55 (32%) of the patients were admitted with mild infections, and corticotherapy may be used after efficient antibiotherapy. Even though infections are common in AH patients, Louvet et al. show that therapy can still be continued and that a patient's reaction to medicine is the most crucial factor in their short-term survival.

In our univariate study, MELD score, MDF, and LM4 were associated with 28-day mortality. LM4's performance did not differ from those of MELD, MADDREY, or LM7 (z-score = 0.58, p = 0.64). A Day 4 Lille score greater than 0.45 increases the chance of death by 7.2 times. Our findings were comparable to other research in that MDF was largely used at the start, but MELD performed better when predicting mortality.

The combination of different scoring systems has proven to be more useful in predicting outcomes for patients with AH. When static indicators like Maddrey, MELD, and ABIC are combined with the dynamic Lille score, predictive accuracy improves. A research by Louvet et al., found that the MELD + Lille score can more accurately predict outcomes of patients with AH. According to our findings, the most accurate model for predicting 28-day

mortality combines the MELD score with either LM4 or LM7, with a high accuracy of 0.90. Combining MELD and MDF yields an accuracy of 0.86, whereas combining LM4/7 with MELD and MDF yields an accuracy of 0.87. This implies that combining these scoring systems improves their prediction ability.

A high level of agreement (91.1%) was found between LM4 and LM7 in predicting the response to corticosteroids. By calculating LM at Day 4 using Day 4 bilirubin instead of Day 7 bilirubin, we demonstrated that LM4 independently predicted mortality at 28 and 90 days in multivariate analysis. Regarding the performance in predicting mortality, the MELD score outperformed other scores in our study, with an AUC of 0.85. Additionally, we demonstrated that LM4 and LM7 had a correlation of r = 0.94, p 0.0001, and R2 = 0.88, which means that 88% of LM4's values agreed with those of LM7.

Diagnosis and prognosis play a crucial role in the management of alcoholic hepatitis. Timely and accurate diagnosis enables the implementation of suitable treatment strategies, while prognosis assessment assists in making informed treatment decisions and predicting patient outcomes. A comprehensive strategy that integrates diagnosis, illness severity assessment, and prognosis evaluation is required for developing treatment programs, tracking disease progression, and engaging patients in shared decision-making.

Highlighting the importance of diagnosis and prognosis in alcoholic hepatitis is vital for achieving appropriate treatment and better outcomes among patients. These findings need to be validated in a larger patient group, although the preliminary results are promising.

GENERAL CONCLUSIONS

1. There is an urgent need for improved strategies for screening and early diagnosis of ALD, particularly for liver fibrosis, which is a strong predictor of disease severity.
2. Screening for ALD in patients with AUD is mandatory, because in our cohort 70.9% presented moderate and severe liver steatosis and 17.5% were newly diagnosed with liver cirrhosis.
3. A proposed algorithm can facilitate the early detection and appropriate management of patients with alcohol-related liver disease, starting from primary care settings.
4. Non-invasive tests, such as indirect serum markers, can be applicable for patients with ALD, making them suitable for primary care settings.
5. Liver stiffness assessed by FIB-4 as a non-invasive biological marker showed good performance for predicting advanced fibrosis.

6. Elastography-based techniques, including VCTE, pSWE, and 2D-SWE, show promising results for non-invasive assessment of liver fibrosis in ALD patients.
7. Liver stiffnesses assessed by means of pSWE and 2D-SWE techniques implemented in the same ultrasound machine showed good performance for the diagnosis of significant fibrosis liver cirrhosis.
8. pSWE and 2D-SWE are feasible methods for liver stiffness assessment strongly correlates with VCTE results
9. The optimal cut-off values for significant liver fibrosis in any etiology by p-SWE and 2D-SWE are >5.9 kPa and >6.1 kPa, respectively.
10. The optimal cut-off values for liver cirrhosis in any etiology by p-SWE and 2D-SWE are >8 kPa and > 7.6 kPa, respectively.
11. Lille score at 4 days is as accurate as LM 7 in predicting response to corticosteroids and 28-day mortality in patients with severe alcoholic hepatitis
12. Combination of MELD plus LM4 or LM7 better predicts mortality at 28 days with accuracy of 0.90
13. The findings regarding LM4 may reduce the chance of corticotherapy-related problems, reduce hospital expenditures for sAH patients, and, most encouragingly, shorten the time needed to evaluate highly selected patients for liver transplant by three days.