Ultrasound Image Computerized Analysis for Non-invasive Quantitative Evaluation of Hepatic Fibrosis

Georgiana Nagy[1], Maria Adriana Neag[2], Mihaela Gordan[3], Doinita Crisan[4], Mircea Petru[1], Romeo Chira[1]

[1]1st Department of Internal Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Department of Pharmacology, Toxicology and Clinical Pharmacology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[3]Technical University of Cluj-Napoca, Romania
[4]Department of Morphopathological, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Cite this article as: Nagy G, Neag MA, Gordan M, Crisan D, Petru M, Chira R. Ultrasound image computerized analysis for non-invasive quantitative evaluation of hepatic fibrosis. *Turk J Gastroenterol.* 2021; 32(10): 888-895.

ABSTRACT

Background: *Assessing the diagnostic value of liver ultrasound image computerized analysis (USICA) for hepatic fibrosis (HF) staging in respect to the "gold standard" provided by liver biopsy (LB).*

Methods: *Two-hundred twenty-eight patients with chronic hepatopathies were prospectively enrolled in the study. All the patients underwent LB and abdominal ultrasound (US). For quantitative US assessment of HF, an image analysis software was developed and 3 parameters were extracted by wavelet processing of the region of interest: mHLlivermHHliver, mHLlivermLLliver, and mHLlivermHL-spleen. To assess the relevance of each feature, the support vector machine (SVM) classifiers were employed to discriminate between the 2 severity classes (i.e., incipient F1-F2 vs advanced F3-F4 fibrosis). The statistical significance of the HF staging was assessed using SVM classifiers, in terms of sensitivity (Se), specificity (Sp), and receiver operating characteristic (ROC) curves.*

Results: *A cut-off value of 0.342 of mHLlivermHHliver allowed the discrimination between the incipient and advanced HF with 79.5% Se and 77.4% Sp, at an area under receiver operating characteristic (AUROC) value of 0.867 ($P < .001$).*

Conclusion: *The proposed USICA using wavelet filter parameters proved to be an innovative method that is useful for the initial non-invasive evaluation and quantification of HF, with the advantages of simplicity, short calculation time, accessibility, and repeatability. The mHLlivermHHliver parameter has demonstrated good accuracy in distinguishing incipient and advanced HF and can be considered an effective non-invasive imaging marker for the assessment of HF in patients with chronic hepatic disease.*

Keywords: *Quantitative liver ultrasound, computer-assisted image analysis, fibrosis, chronic hepatopathies*

INTRODUCTION

Following an acute liver injury, the liver can quickly restore its original architecture. However, chronic injuries that target the liver can cause changes that go beyond the regeneration capacity of this organ. The process of liver regeneration is initially beneficial, but over time, it becomes pathogenic, as normal tissue is replaced by scar tissue which changes the overall architecture of the liver in such a manner that hepatic fibrosis (HF) is installed. Hepatic fibrosis progression influences hepatocyte function and increases intrahepatic resistance to portal flow. Thus, hepatic insufficiency, portal hypertension, and finally hepatic cirrhosis can appear. Chronic hepatic injury may have different etiologies: viral (B, C, D), ethanolic, toxic, or autoimmune hepatitis.[1,2] Therefore, for the proper management and treatment of liver disease, the precise staging of the fibrosis is essential. Advanced fibrosis has been shown to be the major risk factor for long-term outcome and mortality.[3]

Liver biopsy is considered the gold standard for HF quantification, despite its well-known limitations such as invasiveness, poor acceptability, sampling variability, and the risk of complications.[4] Due to those limitations, alternative, non-invasive methods for HF quantification have been developed.[5] Hepatic ultrasonography (US) is the most widely used method for HF evaluation, as it is a simple, reproducible, and a highly accurate method for diagnosing liver cirrhosis.[6] However, despite the various advantages of US, the data obtained are non-specific, polymorphic, and can range, even in the presence of significant HF, from normal ultrasound appearance to changes suggestive of liver cirrhosis.[7]

Corresponding author: **Maria Adriana Neag**, e-mail: **borosmeda@yahoo.com**
Received: **October 31, 2020** Accepted: **January 22, 2021** Available Online Date: **November 1, 2021**

DOI: 10.5152/tjg.2021.20951

Computerized analysis of US images (ultrasound image computerized analysis (USICA)) can identify tissue changes caused by liver disease and can quantify them using specific parameters. There are studies about quantitative analysis of hepatic US which used statistical data on the US echo signal,[8] texture analysis of B-mode images,[9,10] and assessment of the scattering properties and fractal dimension of the scattered signal.[11]

In routine clinical practice, an objective and quantitative method to evaluate HF on B-mode images would be useful for the follow-up of patients with chronic liver disease.

OBJECTIVES

Our goal was to identify a suitable computer technique that can analyze images obtained by the USICA, then extract quantitative data, and use the data to identify different stages of liver fibrosis with higher accuracy compared to liver biopsy.

PATIENTS AND METHODS

The study was conducted according to the principles of the Declaration of Helsinki (1964) and its amendments (Tokyo 1975, Venice 1983, Hong Kong 1989). The study was approved by the Ethics Committee of our university and all patients signed an informed consent for their participation in this study.

This study was a prospective study, conducted in a single center. From 2008 to 2011, all patients with confirmed diffuse chronic viral hepatitis C, B, and non-alcoholic fatty liver disease (NAFLD), who were scheduled for liver biopsy, were enrolled in the study. The purpose of liver biopsy was to stage the disease and quantify liver fibrosis according to the METAVIR score. The total number of subjects included in the study was 228.

Patients with detectable HBsAg, anti-HCV in the blood serum, and those with an established diagnosis NAFLD were included in the study. The exclusion criteria were as follows: patients who were unable to give a written consent, had a major cognitive or mental impairment, or were diagnosed with other chronic liver disease (toxic, drug, autoimmune, genetic). Each patient underwent abdominal ultrasound examination (using Megas-Esaote Biomedica ultrasound) following a standardized examination protocol, a day prior to LB. In the study, 2 hepatic and 2 splenic ultrasound sections were performed and saved in "bmp" format for each patient. These sections were stored in a database, for further use in USICA.

Liver Histology Assessment

Ultrasound-guided percutaneous LB was performed with a Tru-cut needle (Bard biopsy gun 16 gauge). An LB specimen of 13 mm containing at least 16 portal tracts was considered adequate for evaluation. All biopsy fragments were blindly examined by the same pathologist who did not know the USICA results.

Liver fibrosis was staged on a 0 to 4 scale according to the METAVIR scoring system,[12] the indications were F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Brunt criteria were used to stage and grade liver steatosis as follows:[13] necroinflammation graded 0 (absent) to 3 (1, occasional ballooned hepatocytes and no or very mild inflammation; 2, ballooning of hepatocytes and mild to moderate portal inflammation; 3, intra-acinar inflammation and portal inflammation).

US Examination and Computerized Image Analysis

An Esaote Megas ultrasound machine (Biomedica, Italy) with 3.5 MHz frequency convex probes was used. The identification and selection of the ultrasound hepatic and splenic sections was performed by placing the transducer in the ninth intercostal space, parallel to the ninth and tenth ribs. These sections were saved as bitmap files in a database. All images were measured by the same examiner using the same ultrasound machine settings. The processing parameters were set to: B/M gain—maximum, the curve "Time Gain Compensation"—minimum, and the examination depth—10 cm, therefore the pre/post-processing curves (PST) were 2/4 and the pulse repetition frequency (PRF) was set to 6.1 kHz. For USICA, we developed a Windows application which allowed automatic localization of a region of interest (ROI) of 1.6/1.6 cm (64×64 pixels), followed by a local feature extraction in the ROI, based on the ROI intensity histogram and the dyadic wavelet transform of ROI, applied in a single decomposition step.

In each of the 4 sub-bands resulting after the wavelet decomposition, the mean of the absolute values of the coefficients from the particular sub-band were computed and denoted as mLL, for the low-frequency horizontal/low-frequency vertical decomposition sub-band (LL sub-band, shown in Figure 1); mHL,for the high-frequency horizontal/low-frequency vertical sub-band (HL sub-band, shown in Figure 1); and mLH and mHH (for the other 2 sub-bands shown in Figure 1, with similar meaning as the first 2 described above). mHLliver indicates

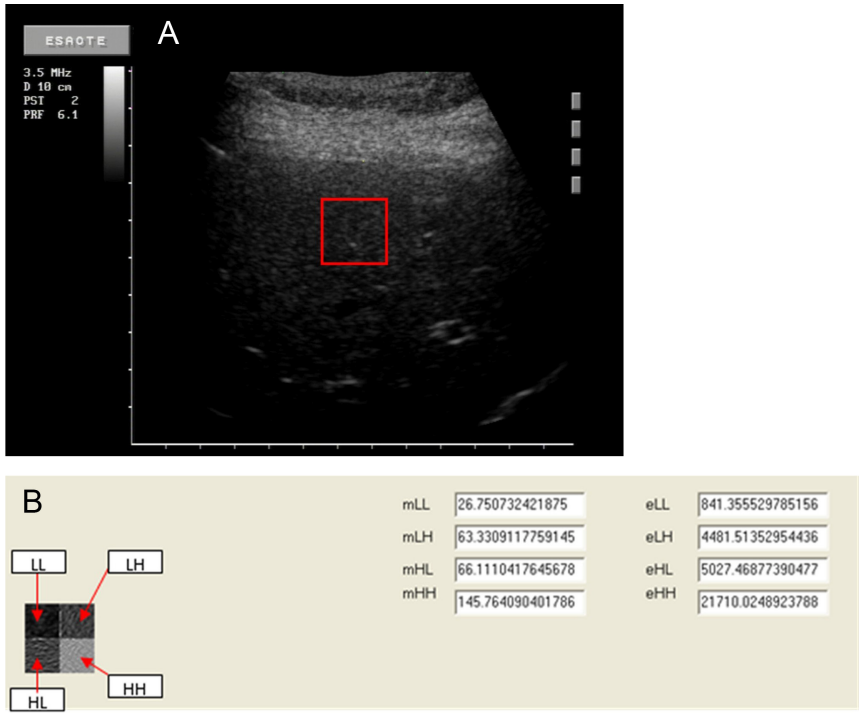

Figure 1. The software interfaces of the Windows application developed for USICA during different processing steps.

that the ROI is taken from the liver ultrasound section, whereas mHLspleen indicates that the ROI is taken from the spleen ultrasound image of the same patient, during the same examination. Since no single feature was discriminative enough between fibrosis stages, but pairs of 2 features were sufficient, we considered the use of several feature pairs, denoted in the following as, for example, mHLlivermLLliver, if the feature itself is a vector with 2 components: [mHLliver mLLliver].

To maximize the hepatic eco-structure changes (fine granularity, heterogenicity), the ROI was placed in the area where the most obvious changes in the US image are observed, avoiding the hepatic subcapsular area and the vascular or biliary landmarks. The most relevant features in terms of strikethrough liver fibrosis description were: mHLlivermHHliver, mHLlivermLLliver and mHLlivermHLspleen. The parameters were extracted by wavelet processing of the ROI, which is equivalent to applying 4 frequency filters on the ROI. Consequently, more structural details are highlighted, which can be significant for fibrosis quantification. (Figures 1A and B).

Figure 1Aand B The software interfaces of the Windows application developed for USICA during different processing steps: after selecting and opening the US image to be analyzed, positioning of ROI with the extraction of the numerical parameters after image processing using the dyadic wavelet filters, and the gray-level histogram Q3

To assess the relevance of each feature, the support vector machine (SVM) classifiers were employed to discriminate between the 2 severity classes (i.e., incipient F1-F2 versus advanced F3-F4 fibrosis). We trained the SVM classifiers on a group of 111 patients (train group) and used the remaining 117 patients as the test group.

Statistical Analysis

The statistical significance of the numerical features extracted to assess liver fibrosis severity was calculated by SVM classification. We used non-linear SVMs with RBF kernel—the most suitable for many medical data classification problems. The classification results were expressed in terms of accuracy, sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV). The diagnostic performance of each feature was also assessed by receiver operating characteristic (ROC) curves; the cut-off value was set to maximize the sum of Se and Sp.

RESULTS

Out of 228 patients (mean age 44 ± 11.38 years, 117 male), 178 had chronic HCV hepatitis. The histopathological Q4

Table 1. Distribution of the Patients Based on the Severity of Fibrosis

Severity of Fibrosis (METAVIR)	No. of Patients (%)
F0	2 (0.87)
F1	51 (22.36)
F2	102 (44.73)
F3	62 (27.19)
F4	11 (4.82)

evaluation revealed that most of the patients examined (67.09%) presented early or mild fibrosis (F1-F2), 44.73% of them had mild HF (F2), whereas only 32.01% of them had advanced HF (F3-F4) (Table 1).

For USICA, we grouped the patients with HF F1-F2 into incipient HF and the patients with HF F3-F4 into advanced HF.

To assess the relevance of each feature, we employed SVM classifiers on a set of 111 patients (train group) and used the remaining 117 patients as the test group, to discriminate between the 2 HF severity classes (i.e., incipient vs advanced HF). The selection of the patients for the test, respectively the train group was done automatically and randomly by the computer.

The anthropometric, biological, and histological characteristics of the studied groups are detailed in Table 2.

Table 2. Characteristics of the Studied Groups

	Mean ± Standard Deviation (%)	
Patient Characteristics	Train Group	Test Group
Patients	111	117
Age (years)	43.31 ± 11.32	45.43 ± 9.93
Female	46	66
Male	65	51
AST (U/l)	58.64 ± 37.01	73.65 ± 49.76
ALT (U/l)	85.21 ± 64.96	102.61 ± 72.8
Bilirubin (mg/dL)	0.79 ± 0.36	0.81 ± 0.39
GGT (U/L)	80.96 ± 129.53	85.31 ± 90.74
Glucose (mg/dL)	94.79 ± 15.99	97.46 ± 25.78
Cholesterol (mg/dL)	175.81 ± 39.04	182.56 ± 45.17
Triglycerides (mg/dL)	106.07 ± 46.47	110.81 ± 62.79
Q5 The length of the liver biopsy	13.09 ± 3.16	13.4 ± 3.01
Number of portal spaces	14.85 ± 5.42	16.85 ± 5.46

Table 3. The Classification Accuracy of Hepatic Fibrosis Staging in each Individual Feature Space Using Non-Linear Support Vector Machine Classifiers (SVM)

	Classification Accuracy	
Parameter	Train Group	Test Group
mHLliver mHHliver	100%	78.64%
mHLlivermLLliver	95.5%	77.78%
mHLlivermHLspleen difference	92.8%	74.36%

The overall accuracy of discrimination between the 2 HF classes by each of the extracted features is given in Table 3.

The composite parameter mHLlivermHHliver achieved a good accuracy (78.64%). At an optimal cut-off value of 0.342 for the test group, mHLlivermHHliver allowed the discrimination between incipient and advanced HF with Se of 79.5% and Sp of 77.4%, at an area under receiver operating characteristic (AUROC) value of 0.867 (Table 4).

Figure 2 illustrates the distribution and the variation of the numerical values of the mHLlivermHHliver composite parameter, with a graphical representation of the separation performance between the 2 distinct classes of fibrosis (F1-F2 vs F3-F4) on the train lot, respectively on the whole lot (test + train).

The mHHlivermLLliver parameter is slightly less accurate (77,78%) compared to mHLlivermHHliver (Table 4). Q6
The optimal cut-off value for the prediction of advanced fibrosis was 0.99, with Se and Sp of 67.1% and 65.2% for an acceptable AUROC of 0.705.

Figure 3 illustrates the distribution and the variation of the numerical values of the mHLlivermHHliver composite parameter, with a graphical representation of the separation performance between the 2 severity classes of fibrosis (F1-F2 vs F3-F4) on the train lot, respectively on the whole lot (test + train).

The composite parameter mHLlivermHLspleen difference was considered a weak discriminative parameter, having an accuracy of 74.36%, Se of 57.5% and Sp of 63.6% for a AUROC of 0.757 (Table 4).ROI Q7

Figure 4 illustrates the distribution and the variation of the numerical values of the mHLlivermHLspleen composite parameter, with a graphical representation of the separation performance between the 2 severity classes of

Table 4. Statistical Performance of the Composite Parameters (mHLlivermHHliver, mHLlivermLLliver, mHLlivermHLspleen difference) for Liver Fibrosis Staging (F1-F2 vs F3-F4)

Parameter	mHLlivermHHliver	mHLlivermLLliver	mHLlivermHLspleen difference
Cut-off value	–	0.99	0.03
Se (%) [95% CI]	79.5	67.1	57.5
Sp (%) [95% CI]	77.4	65.2	63.6
PPV (%) [95% CI]	62.4	47.6	35.9
PNV (%) [95% CI]	88.9	80.8	80.9
AUROC [95% CI]	0.867	0.705	0.757

Se, sensitivity; Sp, specificity; PPV, predictive positive value; NPV, negative predictive value; AUROC, Area Under Receiver Operating Characteristic Curve; 95% CI, confidence interval.

fibrosis (F1-F2 vs F3-F4) on the train lot, respectively on the whole lot (test + train).

The presence and the severity of hepatic steatosis did not influence the stage of liver fibrosis (P = .42 for mild steatosis vs P = .72 for moderate-severe steatosis), even though approximately 55% of the patients had steatosis.

DISCUSSIONS

For many years, liver biopsy has been considered the gold standard for the diagnosis of chronic liver disease. Currently, as it is an invasive method with the risk of complications, the use of non-invasive techniques is on the rise, for example, ultrasound elastography including transient elastography, acoustic radiation force impulse

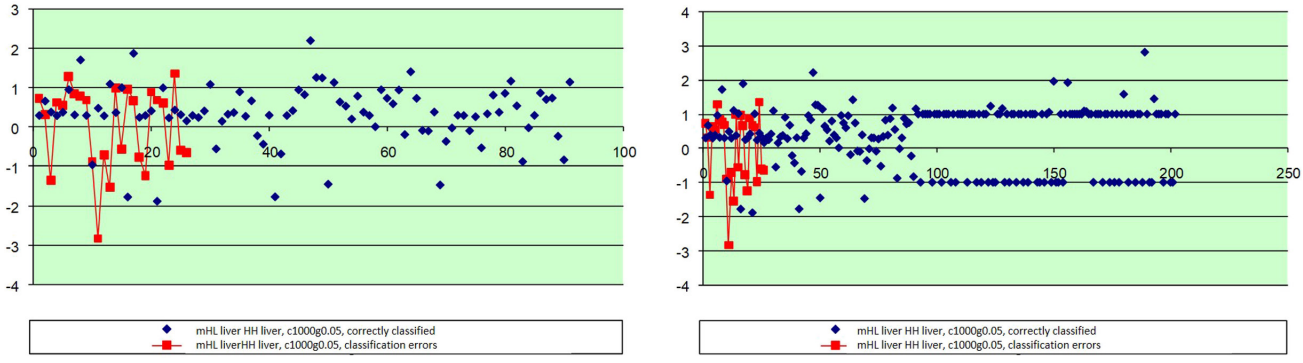

Figure 2. Graphical representation of the separation of the 2 severity classes of fibrosis, using the composite parameter mHLlivermHHliver, on the test lot (left) and on the whole lot (right).

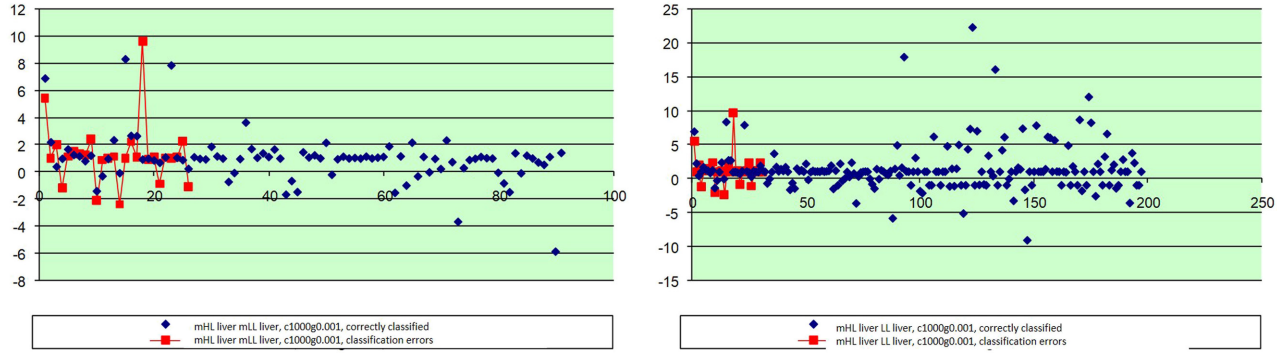

Figure 3. Graphical representation of the separation of the 2 classes of fibrosis severity, using the composite parameter mHLlivermLLliver, on the test lot (left) and on the whole lot (right).

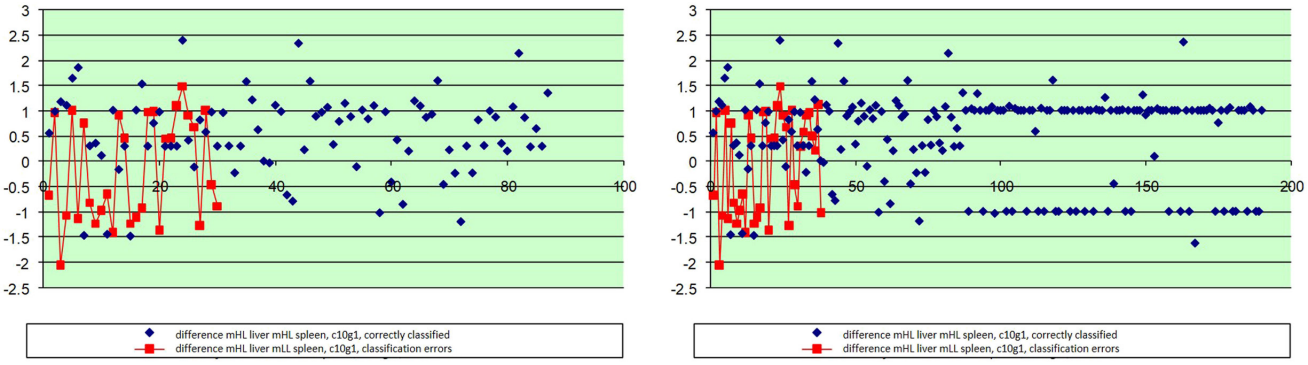

Figure 4. A graphical representation of the separation of the 2 severity classes of fibrosis, using the composite parameter mHLlivermHLspleen, on the test lot (left) and on the whole lot (right).

imaging, shear wave elastography, and magnetic resonance elastography.[14] These methods were demonstrated to provide a reliable evaluation of liver fibrosis.[15] It is well known that the various ultrasound elastography methods have mutual limitations, for example: liver stiffness is influenced by inflammation. Furthermore, they are operator-dependent and a specific probe and system are required for clinical application. Moreover, their clinical application is substantially limited by obesity, ascites, and the high rate of uninterpretable results.[16-18]

In view of the fact that these methods are not always available in the current clinical practice, using a computer-aided ultrasound diagnostic method for yielding liver fibrosis-related information becomes crucial.

Our study proposes an innovative method using simple features based on the analysis of hepatic textural changes, obtained by processing ultrasonographic images with wavelet filters by computerized quantitative ultrasonographic analysis, using non-linear SVM classifiers. The composite parameter mHLlivermHHliver achieved a good accuracy (78.64%) in distinguishing the 2 previously established classes of fibrosis severity (incipient vs advanced HF). On the other hand, the mHHlivermLLliver parameter had a slightly inferior performance at classifying the 2 distinct classes of HF.

Although the performance of ultrasound elastography methods for the evaluation of fibrosis is considered better than the method proposed by us, our method has several other advantages over the classic transient elastography, as it provides a real-time image of the liver and allows ROI positioning—which can help to avoid focal lesions, large vessels, and the interference of ascites and obesity—factors that commonly limit the transient elastography examination.[17]

Data obtained from the train group show that SVM is highly accurate in distinguishing incipient fibrosis (F1-F2) from advanced fibrosis (F3-F4). In our group, most of the participants had portal fibrosis with few septa (F2), whereas only 2 of them had no fibrosis (F0). Discrepancy between our classification system for fibrosis and the histopathological staging was most observed at F2. Therefore, this was the reason for grouping F1-F2 and F3-F4 and investigating the parameters in the US images to differentiate between the incipient and advanced stages.

In fact, SVMs are very powerful classical machine learning classifiers which yield very good results in various liver diagnosis systems—imaging and serum biomarker-based[19]—being recently reported in some reviews for their performance.[20,21] However it is worth noting that, according to the studies, the extraction of relevant features from ultrasound images for fibrosis staging is the most difficult/often unsuccessful using classical machine learning alone (for such situations, the combination of deep learning approaches—as convolutional neural networks—with SVM classifiers seems a better solution). An SVM-based approach more similar conceptually to ours is the one proposed by Virmani et al.[22] They used a CAD system based Q8
on B-mode liver US images to differentiate between normal liver, liver cirrhosis, and hepatocellular carcinoma. Their results showed an overall classification accuracy of 88.6%, with Se of 90% for normal liver and cirrhosis and 86.6% for liver HCC.[22] Even though their results were significantly better than ours, their study was limited by the small number of subjects included and they did not use LB as a diagnostic method. Moreover, parameters were

used to differentiate between normal and cirrhotic liver and liver tumors, where structural changes of the liver are clearly notable using 2-dimensional ultrasound.

For the mHLlivermHHliver parameter, the optimal cut-off value was 0.342 in the test group and 0.99 for the mHHlivermLLliver group. These values allow the differentiation between incipient (F1-F2) and advanced fibrosis (F3-F4) with Se and Sp of 79.5% and 77.4% respectively, versus 67.1% and 65.2% respectively, but with good NPVs of 88.9%, and 80.8% respectively. The high heterogeneity of the group in terms of the etiology of chronic liver disease (B, C viral hepatitis, NASH) led to an unsatisfying performance in determining the classes of fibrosis severity. It is well known that the fibrogenesis model is variable for the same stage of fibrosis, as it depends on the etiology of liver disease.

Huang et al.,[23] using acoustic structure quantification (ASQ), obtained better results in differentiating the stages of fibrosis (F2, Se = 81.9%, Sp = 80%; F3, Se = 91.5%, Sp = 65.5%; F4, Se = 69.2%, Sp = 85.3%). As a term of comparison, they enrolled a smaller number of patients than us (about a half) and included only 1 type of chronic liver disease (HBV hepatitis). Furthermore, the histopathological examination was performed by 2 pathologists using hematoxylin–eosin staining which can lead to errors in staging the severity of fibrosis.[23] Moreover, the method used to analyze images was quite complex and required expensive devices.

In another study, Tsui et al.,[24] using ASQ and Nakagami ultrasound US imaging to assess the stage of fibrosis, obtained better results in distinguishing the early stage of fibrosis (F1, Se = 75.8%, Sp = 91.3%; F2, Se = 85.29%, Sp = 76.19%), however, they obtained poor results in staging advanced fibrosis (F3, Se = 71.11%, Sp = 64.62%; F4, Se = 72.73%, Sp = 59.74%).[24] In Tsui and Huang's research, ultrasound image analysis was performed on the entire image, aiming to observe the heterogeneity of the liver.[23,24]

In the proposed analysis, we only used a small ROI, therefore the computational complexity of our approach was significantly lower. Unlike the studies presented earlier, the ultrasound device that we used does not have a software for ASQ computation; hence, better results in assessing the homogeneity of the liver structure are expected from our study if we use an ASQ computation software for processing our data.

In addition to non-invasive imaging techniques for evaluating the fibrosis of chronic hepatopathies, especially of non-alcoholic fatty liver disease (NAFLD), the guidelines[25] and the recent studies recommend using non-invasive serological tests such as FIB-4, NFS, and FibroTest, alone or in combination with the imaging techniques (FibroScan) for a more precise quantification of the HF. Recently, Jafarov et al.[26] and Kaya et al.[27] have shown that by utilizing the combination of FIB-4 with unidimensional transitory elastography on NAFLD patients, they could predict advanced stages of fibrosis (F≥3) with an 89% sensitivity and an 82% specificity. From the perspective of these studies, a future research direction that we will consider is utilizing USICA in combination with serological biomarkers (non-invasive serum biomarkers/scores) for the evaluation of HF in patients with chronic hepatopathies, aiming for improved diagnostic accuracy.

Our study was limited by the considerable difference between the number of patients included in the incipient fibrosis group and the ones included in the advanced fibrosis group. Patients with incipient fibrosis accounted for 67.96% (two-thirds of the total number of patients). There was a small number of patients with advanced fibrosis who served for initialization of the SVM system, which could have had a negative impact on the fibrosis classification (F1-F2 vs. F3-F4). Also, we did not use these parameters to monitor the same patient over time, nor did we compare the results using one-dimensional transient elastography.

CONCLUSIONS

Ultrasound image computerized analysis using wavelet filter parameters proved to be an innovative method that is useful for the initial non-invasive evaluation and quantification of liver fibrosis. Furthermore, its several advantages, including simplicity, short calculation time, accessibility, and repeatability can substantially ease HF evaluation in patients with chronic liver disease. The mHLlivermHHliver parameter has demonstrated satisfactory accuracy in distinguishing incipient and advanced fibrosis and can be considered an effective non-invasive imaging biomarker for the assessment of liver fibrosis in patients with chronic hepatic disease. This software application can be incorporated into the conventional US system and can be used during routine US examination of the liver, especially for monitoring the time course of fibrosis in the same patient.

Ethics Committee Approval: The study was approved by the Iuliu Hatieganu Unviersity of Medicine and Pharmacy ethics committee, nr. 1414/2006.

Informed Consent: At the time of admission, all patients signed an informed consent regarding their data processing.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – G.N., M.G., M.P.; Design - G.N., M.G.; Supervision - M.A.N., M.P., R.C.; Resource - D.C., R.C.; Materials - D.C.; Data Collection and/or Processing - G.N., D.C.; Analysis and/or Interpretation - G.N., M.G.; Literature Search - G.N., M.A.N., M.G., D.C.; Writing - M.A.N., M.G.; Critical Reviews - M.P., R.C.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Sauerbruch T, Schierwagen R, Trebicka J. Managing portal hypertension in patients with liver cirrhosis. F1000Research. 2018;7: F1000. [CrossRef]
2. Zhang CY, Yuan WG, He P, Lei JH, Wang CX. Liver fibrosis and hepatic stellate cells: etiology, pathological hallmarks and therapeutic targets. World J Gastroenterol. 2016;22(48):10512-10522. [CrossRef]
3. Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. Hepatology. 2017;65(5):1557-1565. [CrossRef]
4. Rockey DC, Caldwell SH, Goodman ZD, et al. Liver biopsy. Hepatology. 2009;49(3):1017-1044. [CrossRef]
5. Schwenzer NF, Springer F, Schraml C, et al. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. J Hepatol. 2009;51(3):433-445. [CrossRef]
6. Pinzani M, Rombouts K, Colagrande S. Fibrosis in chronic liver diseases: diagnosis and management. J Hepatol. 2005;42(1)(suppl):S22-S36. [CrossRef]
7. Lurie Y, Webb M, Cytter-Kuint R, Shteingart S, Lederkremer GZ. Non-invasive diagnosis of liver fibrosis and cirrhosis. World J Gastroenterol. 2015;21(41):11567-11583. [CrossRef]
8. Fujii Y, Taniguchi N, Wang Y, et al. Clinical application of a new method that segments the region of interest into multiple layers for RF amplitude histogram analysis in the cirrhotic liver. J Med Ultrason (2001). 2004;31(2):91-98. [CrossRef]
9. Gaitini D, Lederman M, Baruch Y, et al. Computerised analysis of liver texture with correlation to needle biopsy. Ultraschall Med. 2005;26(3):197-202. [CrossRef]
10. Cao GT, Shi PF, Hu B. Liver fibrosis identification based on ultrasound images captured under varied imaging protocols. J Zhejiang Univ Sci B. 2005;6(11):1107-1114. [CrossRef]
11. Yamada H, Ebara M, Yamaguchi T, et al. A pilot approach for quantitative assessment of liver fibrosis using ultrasound: preliminary results in 79 cases. J Hepatol. 2006;44(1):68-75. [CrossRef]
12. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology. 1996;24(2):289-293. [CrossRef]
13. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011;43(8):617-649. [CrossRef]
14. Amarapurkar D, Amarapurkar A. Indications of liver biopsy in the era of noninvasive assessment of liver fibrosis. J Clin Exp Hepatol. 2015;5(4):314-319. [CrossRef]
15. Paparo F, Corradi F, Cevasco L, et al. Real-time elastography in the assessment of liver fibrosis: a review of qualitative and semi-quantitative methods for elastogram analysis. Ultrasound Med Biol. 2014;40(9):1923-1933. [CrossRef]
16. Berzigotti A, Castera L. Update on ultrasound imaging of liver fibrosis. J Hepatol. 2013;59(1):180-182. [CrossRef]
17. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. Gastroenterology. 2012;142(6):1293-1302.e4. [CrossRef]
18. Castera L, Friedrich-Rust M, Loomba R. Noninvasive assessment of liver disease in patients With nonalcoholic fatty liver disease. Gastroenterology. 2019;156(5):1264-1281.e4. [CrossRef]
19. Yilmaz Y, Eren F. Identification of a support vector machine-based biomarker panel with high sensitivity and specificity for non-alcoholic steatohepatitis. Clin Chim Acta. 2012;414:154-157. [CrossRef]
20. Craxì L Letter to the Editor. Reply to Ahn JC, Connell A, Simonetto DA, Hughes C, Shah VH. The application of artificial intelligence for the diagnosis and treatment of liver diseases. Hepatology 2020. [CrossRef]
21. Zhou LQ, Wang JY, Yu SY, et al. Artificial intelligence in medical imaging of the liver. World J Gastroenterol. 2019;25(6):672-682. [CrossRef]
22. Virmani J, Kumar V, Kalra N, Khandelwal N. SVM-based characterization of liver ultrasound images using wavelet packet texture descriptors. J Digit Imaging. 2013;26(3):530-543. [CrossRef]
23. Huang Y, Wang Z, Liao B, et al. Assessment of liver fibrosis in chronic hepatitis B using acoustic structure quantification: quantitative morphological ultrasound. Eur Radiol. 2016;26(7):2344-2351. [CrossRef]
24. 'Tsui PH, Ho MC, Tai DI, et al. Acoustic structure quantification by using ultrasound Nakagami imaging for assessing liver fibrosis. Sci Rep. 2016;6:33075. [CrossRef]
25. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328-357. [CrossRef]
26. Jafarov F, Kaya E, Bakir A, Eren F, Yilmaz Y. The diagnostic utility of fibrosis-4 or nonalcoholic fatty liver disease fibrosis score combined with liver stiffness measurement by FibroScan in assessment of advanced liver fibrosis: A biopsy-proven nonalcoholic fatty liver disease study. Eur J Gastroenterol Hepatol. 2020;32(5):642-649. [CrossRef]
27. Kaya E, Bakir A, Kani HT, et al. Simple noninvasive scores are clinically useful to exclude, not predict, advanced fibrosis: A study in Turkish patients with biopsy-proven nonalcoholic fatty liver disease. Gut Liver. 2020;14(4):486-491. [CrossRef]

Author Queries

JOB NUMBER: 20200951

JOURNAL: TJG

Q1 Please note the minor edit to the title.
Q2 The author has used US for both ultrasonography and ultrasound. Only Ultrasonography is abbreviated here. I have tried to retain ultrasound in expanded form. Please verify whethr correct.
Q3 Pleasea verify whether this is a placeholder. If it is part of text, it can be edited to „ B show the software interfaces....
Q4 Please provide the expansion of HCV.
Q5 Please indicate the units for this parameter in parentheses here.
Q6 Please verify whether this should read (77% or 78%), or (77.78%)
Q7 Please check this.
Q8 Please provide the expansion of CAD.
Q9 Please provide the expansion of NASH.
Q10 Please check the usage of hyphen here.