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Real-time Elastography (RTE) for assessment of liver fibrosis in patients with chronic liver diseases

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Abstract: ***The assessment of the degree of liver stiffness is important in the treatment of liver diseases. The various types of ultrasound elastography are relatively well studied. Transient elastography (TE) is a proven method of assessment of liver stiffness and possesses the properties of a prognostic indicator. In contrast to this method, the significance of strain elastography used to assess the degree of liver stiffness remains insufficiently established.***

Methods. RTE elastography was conducted in 246 patients. 34 of them were with chronic viral Hepatitis C, 80 with chronic viral Hepatitis B, 30 with nonalcoholic liver disease, 30 with alcoholic liver disease, 42 with hepatic cirrhosis and there was a control group of 30 healthy individuals. The biomarkers APRI, Fibroindex, Forn's index, FIB-4, Fibrotest were examined. In all patients without the control group a liver biopsy was performed for histological evaluation of fibrosis. The RT-generated elastographic imaging was subjected to qualitative analysis by a specially developed program and the derived Liver Fibrosis Index (LFI) was compared to histological and laboratory data.

Results. The value of LFI increases as fibrosis progresses. LFI is significantly different in the cases of moderate fibrosis (F0-2) and advanced fibrosis (F3, 4). LFI shows a good correlation in determining advanced fibrosis and good reproducibility of the results. LFI was found to be an independent prognostic factor in patients with chronic liver disease.

Conclusion. Strain elastography can be used to determine advanced liver fibrosis without influence of hepatic inflammation, unlike other serology markers of liver fibrosis. RTE is probably a prognostic factor in chronic liver diseases..

Keywords: Real time liver elastography; strain liver elastography; liver stiffness measure**.**

1. Introduction

The assessment of liver stiffness is essential for the treatment of patients with chronic liver diseases. This is due to the fact that the stiffness caused by the progression of hepatic fibrosis is closely related to the prognosis of chronic liver diseases [13]. Liver biopsy is the gold standard in the assessment of liver fibrosis [2]. However, this is an invasive method that shows that there are possible shortcomings, such as errors in the procedures and variability in the results of different researchers [10, 15]. Therefore, considerable effort is being made to develop non-invasive markers that reflect liver stiffness. Different blood markers and serum models based on an algorithm, such as FIB4 or AST to Platelet Ratio Index (APRI) are used to assess the degree of hepatic fibrosis. Good outcomes of liver fibrosis prediction are then reported [11]. However, similar blood markers may be affected by a variety of factors, regardless of whether or not there is relation to the liver [4].

On the other hand, elastography can be developed as a procedure that is able to assess the stiffness of the liver in a non-invasive way. Most of the methods are costly and special equipment is required for their application. In contrast, RTE can be performed by using a conventional ultrasonic probe during a routine ultrasound scan and RTE has proven effectiveness even in patients with ascites [7]. Several studies also show the effectiveness of RTE in the assessment of hepatic fibrosis in patients with chronic liver diseases [9,14,16,19]. RTE is considered to be a relatively efficient and easy to apply method, but further studies are still needed to provide more evidence and to introduce a standardized method of study [4, 8].

In this study, we assessed the effectiveness of RTE in a contingent of patients with varying degrees of hepatic fibrosis.

2. Methods

Patients

246 patients were examined for the period from 2013 to 2016, who had passed through the Gastroenterology Clinic at the Kaspela University Multiprofile Hospital for Active Treatment. 34 of them were with chronic viral Hepatitis C, 80 with chronic viral Hepatitis B, 30 with nonalcoholic steatosis, 30 with alcoholic hepatitis, 42 with hepatic cirrhosis with HBV or HCV genesis and there was a control group of 30 healthy individuals. In all patients, serological tests for non-invasive biomarkers and RT elastography, followed by a liver biopsy, were performed within 2 days. In the healthy individuals, a liver biopsy was not performed. The chronic viral hepatitis has been proven by positivation of the viral markers HBsAg, Anti-HBcore TOTAL or Anti-HCV, as the patients have entered the Clinic after at least 6 months of the first positivation. The criteria for liver cirrhosis are ultrasound criteria: gross hepatic structure, portal hypertension, ascites, laboratory indicators – thrombocytopenia, hypoalbuminemia, hyperbilirubinaemia. The criteria for non-alcoholic liver disease are ultrasound evidence of steatosis and no medical history of alcohol intake. AST and ALT transaminases are either within the norm or with a slight increase and the viral markers are negative. Patients with alcoholic liver disease reported excessive alcohol abuse /over 140 g pure alcohol per week/, hepatic blood tests show moderate to severely elevated AST and ALT, viral markers are negative.

The control group consists of healthy individuals with normal levels of liver enzymes, negative viral markers, no medical history of cardiac, pulmonary and neoplastic diseases and no excessive alcohol intake (up to 15g of pure alcohol/day on average monthly). This retrospective study has been approved by the institutional ethics committee. Written informed consent was obtained by all patients included in this study.

Measuring the stiffness of the liver

An Aloka Alpha 7 ultrasound system, Hitachi-Aloka, Japan, with an additional elastography module installed, is used for the assessment of liver stiffness by RTE. The transducer model is UST-5412, 5-13MHz. The reception of RT elastogram is in accordance with the manufacturer's protocol and the guidelines published by the World Federation for Ultrasound in Medicine and Biology (WFUMB) [4]. The transducer is placed in the right intercostal space around the 5-8 rib between the front and the middle axillary line. The patients are examined in a lying position, with the right hand raised above the head. The depth of the study is between 20 and 50 mm, with an area of 350 to 500 mm^2. The results are assumed to be exact at a pressure value of 3-4 in green color at a scale of 0 to 6. Liver Fibrosis Index /LFI/ presented in 2013 by Fujimoto et al [5] was used for the comparison of the RTE images.

Histological assessment of liver stiffness

Disposable biopsy guns with tru-cut needle 16Ga, 22mm biopsy length, were used for histological assessment of hepatic fibrosis. The right lobe in the intercostal space was biopsied under ultrasound control after evaluation for the safest and best access. The biopsy was evaluated to be successful in histological data for the presence of at least 5 portal spaces. The histological staging of the degree of fibrosis is calculated using the Metavir scoring system. [1].

Other markers for assessment of liver stiffness

We used the biomarkers APRI, Fibroindex, Fibroscore, Forn's index, FIB-4 and Fibrotest, for the calculation of which Alfa 2 Macroglobuline, Haptoglobin, Apolipoprotein A1, GGT, ASAT, ALAT, total bilirubin, platelets, cholesterol and fasting glucose were examined. Data was collected for age, gender and BMI of the patients. The blood samples were taken on the same day of the RTE.

Statistical analysis

Statistical analysis data obtained from the patients was collected in a Microsoft Excel file. For a statistical study of quantitative variables, the mean and standard deviations were calculated. The diagnostic performances of liver stiffness measurements and of the serologic tests were assessed by using the area under the receiver operating curve (AUROC). ROC curves were thus built for the detection of significant fibrosis (F ≥ 2 Metavir) and cirrhosis (F4). Optimal cut-off values were chosen to maximize the sum of sensitivity (Se) and specificity (Sp). Positive predictive values (PPV), negative predictive values (NPV), positive likelihood ratios (+LR) were also assessed. We calculated 95% confidence intervals (CI) of the AUROC curves to compare their predictive values. We also evaluated the correlation between the non-invasive tests and the histological severity of fibrosis. Statistical analysis was performed using Microsoft Excel and SPSS software, version 19.0 (SPSS).

3. Results

Characteristics of the patients

216 patients with chronic hepatic impairment and mean age of 52.73, of whom 137 men and 79 women were included. The patients with cirrhosis of the liver were 42, with chronic viral Hepatitis B – 80, with chronic viral Hepatitis C – 34, with alcoholic hepatitis – 30 and with nonalcoholic steatosis disease – 30. 30 patients without hepatic impairment were included as a control group. Other causes, such as autoimmune hepatitis, primary biliary cholangitis or liver damage due to the use of narcotic drugs, are rare diseases and a statistically significant number of cases was not achieved. The histological assessment of liver fibrosis was determined in 216 patients under the METAVIR classification (F0 – 70 cases, F1 – 44 cases, F2 – 36 cases, F3 – 24 cases, F4 – 42 cases).

Receiving an elasticity index

Liver Fibrosis Index /LFI/ presented in 2013 by Fujimoto et al. [5] was used for the comparison of the RTE images.

Correlation between the LFI value with histological assessment and biomarkers by diseases

Chronic viral Hepatitis B

• *Elastography/biomarkers*

In the study of the relationship between the laboratory parameters and the Elastography, significant correlation dependence was found only in Fibrotest – 0.0552. (Table 1)

	RT Elasto graphy	Fibro test	APRI	Fibro index	Forns index	FIB-4 score
Correlation Coefficient	1.000	0.552	0.273	0.420	0.434	0.385
Sig. (2-tailed)	.	0	0.014	0	0	0

Table 1

• *Elastography/biopsy*

From the table presented we cannot interpret the results for F1 and F2. This is because, at the F1 stage the area under the curve is less than 0.50 and in the F2 stage there is no significance of the obtained result P = 0.276. In stage F ≥3, AUROC is 0.962 and the diagnostic value shows a threshold level of 24.96, a sensitivity of 100%, a specificity of 89%, a positive prognostic value of 70.8% and a negative prognostic value of 100%. (Table 2)

Stage	Area	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
F1	0.310	0.058	0.005	0.197	0.423
F2	0.593	0.062	0.276	0.471	0.715
F ≥3	0.962	0.020	0.000	0.922	1.001

Table 2

The data presented shows that the study has high sensitivity and specificity values, which means that the test methodology has very good demarcation capabilities and can serve to identify the group of individuals with advanced fibrosis. The same applies to the positive and negative prognostic values. We divided the group into two according to the established threshold and we found that 70.8% of all those with values above 24.96 fall into stage F ≥3 and the others are in stages from F0 to F3. 100% of all at this stage were adequately

recognized, which corresponds to the abovementioned sensitivity P<0.001 ($\chi 2 = 51.32$). (Table 3)

• *Comparison between work and control groups*

	Group	N	Mean	Std. Deviation	Std. Error Mean	u	P
Elastography	Work	80	20.34	9.06	1.01	3.25	<0.01
	Control	30	16.36	3.78	0.68		
APRI	Work	80	0.25	0.23	0.02	5.73	<0.001
	Control	30	0.09	0.03	0.01		
Fibroindex	Work	80	1.09	0.62	0.07	3.28	<0.01
	Control	30	0.74	0.42	0.08		
Forns' index	Work	80	5.17	2.00	0.22	1.60	>0.05
	Control	30	4.66	1.23	0.23		
Fib-4	Work	80	1.75	1.48	0.16	1.75	>0.005
	Control	30	1.40	0.62	0.11		
Fibrotest	Work	80	1.22	1.11	0.12	4.06	<0.001
	Control	30	0.57	0.57	0.10		

Table 3

We established a difference between the two groups on all biomarkers tested, except for forns.index (Table 7). We searched for the relationship between the groups and the established thresholds for Elastography and the Fibrotest score. The entire control group had a value of less than 2 for the Fibrotest score P <0.05 (x2 = 4.41) and over 24.96 for Elastography P <0.01 (x2 = 7.90).

Chronic viral Hepatitis C

• *Elastography/biomarkers*

We established a moderate correlation between Elastography and the Fibrotest score P <0.01 (r = 0.480) and between Elastography and the Forns index P <0.01 (r = 0.427) (Table 4)

	RT Elasto graphy	Fibro test	APRI	Fibro index	Forns index	FIB-4 score
Correlation Coefficient	1,000	0,480	0,209	0,320	0,427	0,224
Sig. (2-tailed)	.	0,005	0,243	0,070	0,013	0,210

Table 4

• *Elastography/biopsy*

It can be seen from the table below that the increase in the degree of fibrosis also increases the Elastography values P<0.001 (F = 11.89). The difference is the most distinct between F0 and F3 stages. (Table 5)

Elastography					95% Confidence Interval for Mean	
METAVIR	N	Mean	Std. deviation	Std. error	Lower Bound	Upper Bound
F0	13	13.44	4.13	1.14	10.95	15.94
F1	8	17.83	6.92	2.45	12.04	23.61
F2	6	21.16	7.64	3.12	13.14	29.17
F3	7	29.67	4.01	1.64	25.45	33.88
Total	34	18.86	7.97	1.39	16.03	21.69

Table 5

According to the fibrosis thresholds the AUROC range from 0.50 (95% CI: 0.29 – 0.71) at ≥ F1, at F2 stage – 0.66 (95% CI: 0.46 – 0.86) and at F3 stage – 0.93 (95% CI: 0.83 – 1.02). However, a statistically significant result is only AUROC at F3 P<0.001, as the statistical error is relatively small – 0.047, which is an indicator of high reliability of the given test methodology. The results speak of good differentiation capabilities of the Real-Time Elastography in the advanced stage of fibrosis. Diagnostic accuracy of the Elastography at F3 stage showed a threshold level of 25.65, sensitivity of 83.3%, specificity of 93%, positive prognostic value of 71.4% and negative prognostic value of 96.2%. The data presented in F3 indicates that the study has high sensitivity and specificity values. This supports the test methodology in having very good differentiation capabilities and can serve to identify the group of individuals with advanced fibrosis.

• *Comparison between work and control groups* (Table 6)

	Group		Elastography		
			Upto 25.64	Over 25.65	Total
	Work	count	26	7	33
		% within group	78.8%	21.2%	100%
	Control	count	19	0	19
		% within group	100%	0%	100%
Total		count	45	7	52
		% within group	86.5%	13.5%	100%

Table 6

There is a significant difference between the two groups P<0.05 ($\chi 2 = 4.66$). All control groups fall into the group below the Elastography threshold of 25.64 (Table 6). This means that Elastography cannot be used and has no diagnostic value in relation to healthy individuals.

Alcoholic liver disease

• *Elastography/biopsy*

When constructing the ROC curves for the respective stages of the biopsy, we established that the analysis had a predictive value only for F2 P <0.001. (Table 7)

Stage	AUROC	Std.error	P	95% Confidence interval	
F1	0.403	0.115	>0,05	0.179	0.628
F2	0.848	0.077	<0,001	0.698	0.998

Table 7

The area under the curve at F2 is AUROC = 0.848, 95% CI (0.698 – 0.998), P <0.001. In this case, it was found that the Cut off value (threshold value) for diagnosing fibrosis in F2 was 17.32, at a sensitivity of 0.810 and specificity of 0.998. The positive prognostic value is 86.7% and the negative prognostic value is 80%.

• *Elastography/biomarkers*

The dependence between the laboratory parameters and the elastography was studied. We established a moderate correlation between the elastography with APRI and fib-4score. (Table 8)

	RT Elasto graphy	Fibro test	APRI	Fibro index	Forns index	FIB-4 score
Correlation Coefficient	1.000	0.294	0.418	0.360	0.309	0.451
Sig. (2-tailed)	.	0.115	0.022	0.051	0.097	0.012

Table 8

• *Comparison between work and control groups*

When we compared the work group with the control group it was established that there was no significant difference between the two groups with respect to the threshold value: P> 0.05 ($\chi 2 = 0.48$) and that we cannot apply elastography as a reliable diagnostic method for this disease.

Nonalcoholic liver disease

• *Elastography/biopsy*

The liver biopsy of all 30 patients established a histological result for the fibrosis stage F0. Therefore, we believe that there is no need for a liver biopsy in this group.

• *Elastography/biomarkers*

The dependence between the elastography and the laboratory biomarkers was studied. Elastography does not correlate with any of the other parameters P> 0.05 (Table 3). The lack of a statistical relationship between them necessitates further studies in this specific patient group. (Table 9)

	RT Elasto graphy	Fibro test	APRI	Fibro index	Forns index	FIB-4 score
Correlation Coefficient	1.000	0.086	0.191	0.084	0.219	0.082
Sig. (2-tailed)	.	0.653	0.313	0.659	0.246	0.667

Table 9

• *Comparison between work and control groups*

When we compared the work group with the control group it was established that there is a significant difference only in the Elastography, the Fibrotest and the APRI, which means that only these indicators should be used in this disease. (Table 10)

Indicator	**Group**	**Pcs .**	**Mean**	**Std. deviation**	Std. Error Mean	u	P
Elastography	Work	30	13.79	5.36	0.98	2.15	<0.05
	Control	30	16.37	3.78	0.69		
Fibrotest	Work	30	1.03	0.99	0.18	2.22	<0.05
	Control	30	0.57	0.57	0.10		
APRI	Work	30	0.25	0.26	0.05	3.25	<0.01
	Control	30	0.09	0.03	0.01		

Table 10

Liver cirrhosis

• *Elastography/biomarkers*

The relationship between Elastography and biomarkers was studied and no correlation was established between them. (Table 11)

	RT Elasto graphy	Fibro test	APRI	Fibro index	Forns index	FIB-4 score
Correlation Coefficient	1.000	0.258	0.168	0.105	0.087	0.181
Sig. (2-tailed)	.	0.099	0.288	0.507	0.583	0.251

Table 11

• *Elastography/biopsy*

We determined a diagnostic accuracy of Elastography in F4 /cirrhosis/ and the method showed a threshold level of 24.67, sensitivity of 85.4%, specificity of 96.8%, positive prognostic value of 85.4% and negative prognostic value of 100%.

• *Comparison between work and control groups*

	Groups	N	Mean	Std. Deviation	Std. Error Mean	u	P
RTE	Work	42	32.29	7.71	1.19	11.58	<0.001
	Control	30	16.37	3.78	0.69		
APRI	Work	42	0.59	0.48	0.07	6.57	<0.001
	Control	30	0.10	0.03	0.01		
Fibroindex	Work	42	1.77	0.54	0.08	8.61	<0.001
	Control	30	0.75	0.43	0.08		
Fornsindex	Work	42	7.59	4.28	0.66	3.63	<0.001
	Control	30	4.67	1.24	0.23		
Fibrotest	Work	42	2.57	1.016	0.157	10.67	<0.001
	Control	30	0.57	0.568	0.104		
Fib-4 score	Work	42	5.83	4.59	0.71	6.19	<0.001
	Control	30	1.40	0.62	0.11		

Table 12

We established a difference between the two groups in relation to all biomarkers tested, which means that the biomarkers have a significant diagnostic value for liver cirrhosis, upon an absence of such for healthy individuals.

4. Discussion

Within the present study, RTE has proved to be an effective tool in the determination of advanced liver fibrosis. Our results showed higher efficiency of RTE, compared to some blood biomarkers for fibrosis. In previous studies for determination of liver stiffness with RTE, similar results occurred, indicating a good diagnostic benefit if an adequate procedure was performed. ROI with an area of 2.5 x 2.5 cm should be placed deeply in the liver capsule, by avoiding large vessels, in order to produce homogeneous images [5,12,18,19]. Our study was also conducted with the purpose to include a sufficient volume of the hepatic parenchyma according to the RTE guidelines. Our results our comparable to other studies due to the fact that the LFI used as an indicator in this study displayed good correlations with histologically proven fibrosis and other markers for fibrosis. The results suggest that LFI is unable to fully differentiate between mild, moderate and advanced stage of liver fibrosis. The RTE method is capable of assessing liver fibrosis without being affected by inflammatory processes of the liver and jaundice [4]. RTE can be used in patients with ascites [7] and can be a suitable method for determining advanced liver fibrosis.

This study contains several limitations. First of all, the LFI indicator used in this study for determination of liver stiffness is a relative assessment. Until now, there is no unified opinion on the use of a particular algorithm. In the European guidelines for the application of Elastography, there is a proposal for the implementation of further studies on RTE [3, 17]. It is necessary for a standardized analytical method for RTE in future large scale multicenter studies to be defined, bur for sure LFI is able to determine advanced fibrosis in HCV and HBV

patients, but not in ALD/NAFLD patients. The successful RTE depends on the clarity of the B-mode images [19]. In the case of patients with HBV infection, a high degree of irregularity of the hepatic parenchyma is detected in B-mode [6], which may have some impact on LFI upon HBV. Further studies are needed in the case of HBV.

In conclusion, our study has demonstrated that the RTE method with the application of LFI can accurately and reliably determine an advanced stage of liver fibrosis.

References:

[1] Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology. 2003;38:1449–57.

[2] Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001;344:495–500.

[3] Cosgrove D, Piscaglia F, Bamber J, et al. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 2: clinical applications. Ultraschall Med. 2013;34:238–53.

[4] Ferraioli G, Filice C, Castera L, et al. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: part 3: liver. Ultrasound Med Biol. 2015;41:1161–79.

[5] Fujimoto K, Kato M, Kudo M, et al. Novel image analysis method using ultrasound elastography for noninvasive evaluation of hepatic fibrosis in patients with chronic hepatitis C. Oncology. 2013;84(Suppl 1):3–12.

[6] Habu D, Nishiguchi S, Kawamua E, et al. Meshwork pattern is an important risk factor for development of hepatocellular carcinoma in patients with HBV-related chronic hepatitis and cirrhosis. Hepatol Res. 2003;25:166–73.

[7] Hirooka M, Koizumi Y, Hiasa Y, et al. Hepatic elasticity in patients with ascites: evaluation with real-time tissue elastography. AJR Am J Roentgenol. 2011;196:W766–71.

[8] Kan QC, Cui XW, Chang JM, et al. Strain ultrasound elastography for liver diseases. J Hepatol. 2015;63:534.

[9] Koizumi Y, Hirooka M, Kisaka Y, et al. Liver fibrosis in patients with chronic hepatitis C: noninvasive diagnosis by means of realtime tissue elastography—establishment of the method for measurement. Radiology. 2011;258:610–7.

[10] Maharaj B, Maharaj RJ, Leary WP, et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. Lancet. 1986;1:523–5.

[11] Martinez SM, Crespo G, Navasa M, et al. Noninvasive assessment of liver fibrosis. Hepatology. 2011;53:325–35.

[12] Morikawa H, Fukuda K, Kobayashi S, et al. Real-time tissue elastography as a tool for the noninvasive assessment of liver stiffness in patients with chronic hepatitis C. J Gastroenterol. 2011;46:350–8.

[13] National Institutes of H. National Institutes of Health consensus development conference statement: management of hepatitis C: 2002-June 10–12, 2002. Hepatology. 2002;36:S3–20.

[14] Ochi H, Hirooka M, Koizumi Y, et al. Real-time tissue elastography for evaluation of hepatic fibrosis and portal hypertension in nonalcoholic fatty liver diseases. Hepatology. 2012;56:1271–8.

[15] Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol. 2002;97:2614–8.

[16] Shiraishi A, Hiraoka A, Aibiki T, et al. Real-time tissue elastography: non-invasive evaluation of liver fibrosis in chronic liver disease due to HCV. Hepatogastroenterology. 2014;61:2084–90.

[17] Sporea I, Bota S, Saftoiu A, et al. Romanian national guidelines and practical recommendations on liver elastography. Med Ultrason. 2014;16:123–38.

[18] Tatsumi C, Kudo M, Ueshima K, et al. Non-invasive evaluation of hepatic fibrosis for type C chronic hepatitis. Intervirology. 2010;53:76–81.

[19] Yada N, Kudo M, Morikawa H, et al. Assessment of liver fibrosis with real-time tissue elastography in chronic viral hepatitis. Oncology. 2013;84(Suppl 1):13–20