UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA

DOCTORAL SCHOOL

PH.D THESIS

(Summary)

NON-INVASIVE ASSESSMENT METHODS OF HEPATIC FIBROSIS IN CHRONIC HEPATIC DISEASE OF VIRAL ETIOLOGY

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Craiova

2015

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Key words:

Hepatic fibrosis, chronic viral hepatitis B, chronic viral hepatitis C, transabdominal ultrasound, elastography, Fibroscan, ARFI

General Part

(Theoretical Stage)

1. Introduction

Chronic liver diseases affect tens of millions of people worldwide and are a major health problem, not only taking into account the diverse aetiology, but also the progressive evolution and the need for a proper therapeutic attitude. With a rising incidence of viral hepatitis and because of increasing morbidity and mortality due to complications, the early diagnosis and the establishment of antiviral therapy represent a priority for patients.

Chronic B or C viral hepatitis requires specific assessment and monitoring, mainly due to the increased risk of liver cirrhosis and hepatocellular carcinoma.

It is estimated that chronic viral hepatitis C affects worldwide approximately 170 million people, while chronic viral infection B appears to affect more than 400 million people.

2. Hepatic Fibrosis

Hepatic fibrosis represents the excessive accumulation of extracellular matrix in the hepatic parenchyma, known as hepatic scar tissue. The fibrosis process is considered to be a component in the pathogenesis of the hepatic disease because it represents the normal healing response of the liver tissue to various types of aggression. Thus, by recruiting inflammatory cells at the injury area, by increasing the cytokine levels and growth factors, by excessively producing extracellular matrix proteins (ECM), it will lead to the reorganization of liver tissue.

3. Hepatic Biopsy Puncture

Over the past 50 years, HB has been considered the standard criterion for classifying fibrosis [93] because it provides the physician with the necessary information not only on the degree of fibrosis but also on other processes that develop at the hepatic level, such as necrosis, inflammation, steatosis, or the level of copper and iron deposits.

The Metavir scoring system was specifically designed to evaluate hepatic impairment in people infected with hepatitis C. The index includes the sum of the scores assigned to the degree

of inflammatory activity (0-4, where 0 - no activity and 3 or 4 represent severe activity - Table 1. The Metavir score for hepatitis C comes with the advantages that it is relatively simple, it focuses on the non-inflammatory lesions, but it also has an increased sensitivity in the degree of assessment. The Ishak score uses a scalar 7-point system where F0 indicates the absence of fibrosis, F5 is considered to be advanced fibrosis, and F6 represents cirrhosis.

The spread of fibrosis is not uniform, so the specimen obtained by HB represents about 1 / 50,000 of the liver tissue. Therefore, in order to have a quality biopsy that correctly assesses fibrosis, it is necessary the fragment to be long enough and contain as many portions as possible.

HB is safe in general, complications being rare, but potentially lethal. The risks associated with obtaining a hepatic biopsy vary from pain (84%) and hypotension, which are the most common, to more serious complications as peritoneal bleeding (0.5%) and damages to the biliary system. However, the morbidity and mortality rate is significantly low (0.09 to 0.12%).

4. Imaging Methods for Hepatic Fibrosis Evaluation

The Impulse Elastography (IE) is a non-invasive elastography imaging technique capable of determining the elasticity of the hepatic tissue by generating a 50 Hz elastic wave and an ultrasound wave (1500 m/s). The technique has been integrated into a device called Fibroscan (Echosens, Paris, France), which uses a 3.5 Hz transducer (standard M probe) that emits consecutive vibrations for recording the values.

Several Romanian studies considered the following cut-off values to be optimal: for the diagnosis of mild fibrosis (F≥1) the cut-off values were between 4.9 and 5.3 kPa, for significant fibrosis (F≥2 Metavir) between 6,8 and 7,4 kPa, for severe fibrosis (F≥3) between 8.6 and 9.1 kPa, and for cirrhosis (F=4) between 11.8 and 13.6 kPa.

Later, Tsochatzis et al., after analysing 17 studies involving HCV patients and 10 studies involving HBV patients, have established that the cut-off values of the hepatic stiffness determined by IE for predicting different stages of hepatic fibrosis were higher in HCV patients compared to HBV ones. Therefore, the cut-off values for HCV patients for the F=2, F=3 and F=4 predictions were: 7,6 kPa, 10,9 kPa and 15,3 kPa, and in the cases of HBV patients, the cut-off values were: 7 kPa, 8,2 kPa and 11,3 kPa.

ARFI is a relatively new elastography technique that can estimate the tissue stiffness by measuring the speed of the shear wave induced by acoustic radiations, technique used with the

Siemens Acuson S2000 Virtual TouchTM US system (Siemens AG, Erlangen, Germany). By using this technology, the hepatic tissue can be visualised, but also a quantification of the hepatic stiffness can be achieved. The higher the stiffness of a tissue, the higher the speed. ARFI has the advantage of providing an elastography measurement of hepatic rigidity with a conventional ultrasound.

Real-Time Elastography (RT-E) is an imaging technique that directly reveals the physical property of tissues using a conventional ultrasonography probe. The principle of this technique is that, if an elastic medium is compressed with constant axial-directional pressure, it produces tissue deformations. If one or more of the constituent elements of the tissue have different degrees of stiffness, their deformation will be different.

5. Serological Methods for Evaluating Hepatic Fibrosis

For many years, the AST/ALT ratio has been used as a non-invasive method for assessing the severity of chronic liver diseases, including chronic viral C infection. Although some studies have achieved promising results, its performance as a non-invasive fibrosis marker is generally low, especially for the diagnosis of advanced fibrosis.

FibroTest (Biopredictive, Paris, France; FibroSure-LabCorp, Burlington, NC, United States) is a scoring algorithm that uses 5 biochemical markers and 2 clinical parameters validated as a tool for detecting liver fibrosis. This includes alfa-2 macroglobulin, haptoglobin, total bilirubin, apolipoprotein-A, gamma-glutamyl-transpeptidase, with age and gender adjustments. Using a patented algorithm, these parameters are combined in order to obtain a numerical value between 0.0 and 1.0. The result correlates with the Metavir fibrosis score.

APRI is a simple test, that combined the ASAT level with the platelet count to predict significant fibrosis and cirrhosis in HCV patients. APRI was helpful for HBV patients with advanced fibrosis compared to those with a low or moderate degree of fibrosis. When the APRI cut-off value was set at 0.5 for HBV patients, the AUROC value for significant fibrosis was 0.673, and the PPV and NPV were 30% and 87%. In other words, APRI can be used to exclude the presence of significant fibrosis.

The FIB-4 score was initially suggested by researchers in the APRICOT study (AIDS Pegasys Ribavirin International Coinfection Trial) to assess the presence of hepatic fibrosis in HIV/HCV coinfected patients. The study of Sterling et al., which included 832 HIV/HCV

coinfected patients, showed that a FIB-4 cut-off value of > 3.25 had a specificity of 97% for the diagnosis of cirrhosis (AUROC = 0.76). The authors estimated that 71% of the biopsies could have been avoided by using FIB-4 in this group.

Special Part

Objectives

The conducted studies were aimed at evaluating and testing new non-invasive imaging elastography methods for quantifying hepatic fibrosis. The modern ultrasound techniques offer new possibilities for pre-therapeutic diagnosis and evaluation in viral hepatopathy patients, either in the early stage of fibrosis or even in cirrhosis. These methods come to the aid of both the physician and the patient, as it eliminates the risks associated with the hepatic biopsy.

1. Study I – *Quantification of hepatic fibrosis with real-time elastography in viral hepatopathy patients*

The objective of this study was to analyse the performance of real-time elastography as a fast and non-invasive evaluation test for hepatic fibrosis in patients diagnosed with chronic hepatic diseases and to establish the most sensitive elastography parameters for the diagnosis of hepatic fibrosis.

Sixty-three consecutive patients diagnosed with chronic viral hepatitis B or C between January 2014 and December 2014 at the Craiova County Emergency Clinical Hospital were enrolled in the study. The viral hepatitis diagnosis was defined by the presence in the serum of anti-HCV antibodies and hepatitis B surface antigens for more than 6 months.

The exclusion criteria included patients diagnosed with hepatitis of different etiology, including: non-alcoholic liver steatosis, patients with a history of drug or alcohol abuse, and those with cardiorespiratory disorders.

This study included 63 patients, of which 19 were diagnosed with HBV infection, while 44 patients were diagnosed with HCV infection. The reference method used to evaluate hepatic fibrosis was IE, based on its recognition and validation by European guidelines. The most recent cut-off values suggested by Tsochatzis in the meta-analysis for HCV patients were used: for F2,

F3 and F4 the values were 7.6 kPa, 10.9 kPa and 15.3 kPa; respectively, for HBV patients, the used limit values were 7 kPa, 8,2kPa and 11,3 kPa. The fibrosis was classified as follows: 11 patients (17.47%) were classified as F0, 7 patients as F1 (11.11), 9 patients as F2 (14.28%), 11 patients as F3 (17.47%), and 25 patients as F4 (39.68%).

The correlation coefficients between the measurements for each parameter were analyzed with the ANOVA test in order to identify any differences, depending on the fibrosis stage. Thus, SD, % AREA, COMP, Skewness, IDM and Contrast have highly significant differences related to the fibrosis stage ($p < 0.001$), while ASM has only significant differences ($p < 0.05$). As for the Kurtosis, ENT and CORR, there were no significant differences concerning the fibrosis stage ($p > 0.05$). The post-hoc analysis using Fisher`s LSD revealed that, in the MEAN parameter, both F3 and F4 values are smaller than F0, F1, with a statistical significance.

With respect to %AREA, using Fisher`s LSD test, we proved that the F0 and F1 values are significantly lower than the values for F2, F3 and F4, and also that the values for F2 are lower than the ones for F4. Concerning COMP, we showed that the values for F0 and F1 are significantly lower than the values for F3 and F4. As noted in the beginning, there were no significant statistical differences for the Kurtosis and ENT values. Analysing the data for Skewness we found that both F3 and F4 values are higher than F0, F1, and that the F2 values are also lower than the F4 values. Analysing the data for IDM, we noticed that the F3 and F4 values are higher than the F0 and F1 values, and that the F2 values are also lower than the F4 values. For ASM we could only show the significant statistical difference between F4 and F0, or F1.

2. Study II – *Comparison and evaluation of various elastography techniques – RT-E, ARFI in quantifying hepatic fibrosis in patients diagnosed with chronic hepatitis B or C*

The study was conducted to evaluate the performance of RT-E quantitative elastography and ARFI in quantifying hepatic fibrosis in chronic viral hepatitis patients, considering IE as a reference method in the hepatic fibrosis evaluation.

The study was a prospective one and included 159 consecutive patients diagnosed with chronic hepatitis B or C (defined by the presence in the serum of anti-HCV antibodies and hepatitis B surface antigens) and 27 healthy volunteers (subjects with no history of hepatic diseases, with a normal liver and spleen appearance at the ultrasound examination, and normal

biologic tests). The exclusion criteria was: patients diagnosed with hepatic diseases of etiology different than viral infections (autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis, alpha 1-antitrypsin deficiency, or Wilson`s disease), patients with a history of alcohol abuse (alcohol consumption> 20 g/day), patients treated with hepatotoxic drugs and cases where TE, ARFI or RT-E were not technically feasible. An informed consent was signed by each patient. The protocol study was in accordance with the Declaration of Helsinki, being approved by the Local Ethics Committee (no. 110/2014) and submitted to the ClinicalTrials.gov (Identifier: NCT02184000). The serological and imaging evaluations were performed on the same day. The imaging techniques (IE, ARFI and RT-E) were performed by various examiners who were unaware of the results of other techniques.

100 consecutive patients diagnosed with chronic hepatitis B or C were included in this study and 27 healthy volunteers were used as control. The fact that IE is a technique validated for the hepatic fibrosis evaluation led to its use as a reference method. We used the most recent cut-off values suggested in the Tsochatzis meta-analysis [119]. For HCV patients, the cut-off values for F2, F3and F4 were 7.6 kPa, 10.9 kPa and 15.3kPa. For the hepatitis B group, the cut-off values were 7kPa, 8.2kPa and 11.3kPa.

In the present study, the two parameters quantitatively evaluated by using RT-E, has a performance comparable to ARFI in the hepatic fibrosis evaluation. Therefore, the LFI and MEAN had higher accuracy rates in the diagnosis of significant fibrosis (F≥2), of 85% and 84%, in comparison with the rate of 81% by using ARFI. Concerning the hepatic cirrhosis diagnosis, ARFI was slightly higher (precision of 86%), in comparison with the RT-E parameters (precision of 83% and 81%).

The cut-off values of ARFI for predicting significant fibrosis and cirrhosis were comparable to the ones stated in literature: 1.38 m/s and 1.84 m/s. But the RT-E data was more difficult to compare. The published RT-E studies are not exactly consistent, many reporting low RT-E readings in the diagnosis of significant fibrosis and cirrhosis, while other studies describe a better accuracy, the difference between them being based on age, the working method of the device and the measured parameters. If we take into account only the studies using the same measurement technique as the present study, we notice that the LFI parameter has a moderate precision for F≥2 (AUROC 79%, sensitivity 78%, specificity 63%) and F=4 (AUROC 85%, sensitivity 77%, specificity 78%), according to a meta-analysis published in 2015. Therefore, we

confirmed that the LFI performance was higher in the diagnosis of significant fibrosis and slightly reduced in the case of hepatic cirrhosis. As far as we know, it is one of the few European studies that, using one of the newest Hitachi US systems, can establish the RT-E performance in the hepatic fibrosis evaluation, along with ARFI and IE.

Final Conclusions

- Chronic liver diseases affect tens of millions of people worldwide and are a major health problem, not only through the diverse etiology, but also through the progressive evolution and the need for a proper therapeutic attitude;
- It is estimated that chronic viral hepatitis C affects worldwide approximately 170 million people, while chronic viral infection B appears to affect more than 400 million people;
- The prognosis and evolution of chronic viral hepatitis is mainly based on the quantification of the hepatic fibrosis stage;
- The fibrosis process is considered to be a component in the pathogenesis of the hepatic disease because it represents the normal healing response of the liver tissue to various types of aggression;
- The chronic hepatic infections of B or C viral etiology represent the main risk factor in the development of hepatic fibrosis, with the most frequent development of hepatic cirrhosis or CHC;
- The hepatic biopsy is considered the standard criteria for classifying hepatic fibrosis; Although it is a safe procedure in general, there is a risk of complications (pain, hypotension, peritoneal bleeding, damage to neighbouring structures);
- The development of new methods to diagnose and evaluate the hepatic fibrosis evolution has been a major concern in gastroenterology;
- The laboratory tests are an attractive and non-invasive option for the evaluation of hepatic fibrosis, with a whole-liver quantification, which can easily be repeated. The indirect fibrosis markers represent the degree of inflammation and the cellular destruction, cholestasis, the synthesis function of the hepatocytes, hypersplenism, while the direct markers offer a direct measurement of the intravellular matrix components, as well as of the enzymes and their effect;

- The laboratory tests are not yet validated for the dynamic evaluation of the fibrosis process or of the portal hypertension, which is why studying them in different situations is still necessary;
- In the case of chronic hepatic diseases, the imaging techniques play an important role in diagnosing and assessing the severity and progression of fibrosis to cirrhosis;
- The ultrasound techniques are non-invasive, relatively simple and low cost options used in all medical centres for diagnosing and following up patients with chronic hepatic diseases;
- The introduction of elastography as an ultrasound technique has brought a major benefit to the diagnosis of various diseases due to its ability to quantify the elasticity of the target tissue;
- The Impulsion Elastography is an elastography technique recognized by European gastroenterology societies, useful in the diagnosis and staging of hepatic fibrosis, but also in establishing the therapeutic attitude;
- The Real-Time elastography is a new technique with important potential in the hepatic fibrosis evaluation. The study made it possible to individualise the 11 parameters characteristic to the software and quantify the hepatic fibrosis index. The statistical analysis revealed that SD,% AREA, COMP, Skewness, IDM and Contrast had high significant differences ($p<0.001$), while ASM had only significant differences ($p <0.05$).
- The study showed that the determined liver fibrosis index was significant in differentiating the advanced stages of fibrosis, with the MEAN parameter having the major impact in determining the fibrosis degree.
- Real-time elastography has been confirmed as a method that can differentiate between stages of advanced fibrosis, which may contribute to the subsequent choice of therapeutic attitude.
- The Acoustic Radiation Force Impulse technique, similar to the Impulse Elastography, has an important advantage in that it can be performed in patients diagnosed with ascites, but also, due to the view of the whole hepatic parenchyma, the area to be studied can be accurately chosen, thus avoiding possible tumour masses. The cut-off values obtained during the conducted study were similar to the ones stated in literature, 1.38 m/s and 1.84 m/s, with a high precision in predicting hepatic fibrosis;

- Taking into account that the hepatic parenchyma becomes less elastic if the hepatic fibrosis progresses, the average relative value within the study area decreases with the hepatic fibrosis: 113.36 (F0-F1); 104.80 (F2); 97.57 (F3) and 88.22 (F4). The MEAN histogram recorded values significantly lower compared to the fibrosis stage (p KW <0.001); the post-hoc comparison indicated significant differences between F0-F1 vs. F3, F0-F1 vs. F4, and F2 vs. F4. The ARFI values have increased with the fibrosis stage KW (p <0.001) and the post-hoc comparison showed significant differences between F0-F1 vs. F3, F0-F1 vs. F4, and F2 vs. F4.
- In the validation of the fibrosis stage, the real-time elastography had a comparable performance in the hepatic fibrosis assessment. Therefore, LFI and MEAN had a higher accuracy, of 85% and 84%, in the diagnosis of significant fibrosis (F≥2), compared to 81% by using ARFI. In the diagnosis of hepatic cirrhosis, ARFI was superior (precision of 86%), in comparison to the RT-E parameters (precision of 83% and 81%).
- The conducted studies are among the few European studies concerning the imaging evaluation of liver fibrosis.
- These techniques can find applicability as non-invasive techniques both as a diagnostic tool and for screening, after an antiviral treatment or for monitoring the risk of developing hepatocellular carcinoma. They are available on a conventional trasonographic system, are easy to use, cost-effective, and in addition, painless;
- The non-invasive evaluation of fibrosis or hepatic cirrhosis attempts to overcome some of the drawbacks of the hepatic biopsy, especially the procedural risk. These techniques correlate with the hepatic biopsy (F4 or F4<0) or with the fibrosis exclusion (F0 versus F>0). This quality may be useful in current practice and can prevent hepatic biopsy in many cases.
- Assessing the fibrosis progression without an invasive intervention is an important benefit to the patient either by measuring the stiffness of the tissue, or by the possibility of successive monitoring over time.

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