NEW QUANTITATIVE ULTRASOUND BIOMARKERS FOR THE DIAGNOSIS OF CHRONIC HEPATITIS AND FATTY LIVER DISEASE

Ph.D. thesis

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1. Introduction

Chronic hepatitis C (HCV) is a significant cause of liver morbidity worldwide. The standard treatment for HCV is now interferon-free direct-acting antivirals (DAAs), which have significantly higher therapeutic efficiency than previous interferon-based regimens, with an eradication rate as high as 90% in non-cirrhotic patients. However, assessment of liver fibrosis is of high importance prior to therapy, as the diagnosis of advanced fibrosis and cirrhosis can alter the choice of a treatment regimen, the therapeutic response rate, and the post-treatment prognosis in affected patients.

Non-invasive methods are now preferred for the initial assessment of liver fibrosis in HCV-infected patients, with shear wave elastography (SWE) being a universally accepted technique for the staging of fibrosis chronic HCV infection. However, there is limited experience in using elastography for patient follow-up after completing antiviral treatment. The aim of the study is to investigate the changes in liver stiffness (LS) with SWE in chronic HCV-infected patients after treatment with DAAs.

This study has significant clinical implications, as it could help clinicians better evaluate the long-term outcomes of HCV patients treated with DAAs and potentially improve patient surveillance and care.

The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide. Obesity and/ or type 2 diabetes are risk factors for NAFLD, and raised liver enzymes in patients with these metabolic risk factors should prompt non-invasive screening for steatosis, non-alcoholic steatohepatitis (NASH), and fibrosis. NAFLD can progress to NASH, which may lead to more severe liver conditions such as fibrosis, cirrhosis, and hepatocellular carcinoma

(HCC). Therefore, an accurate evaluation of liver fat content is crucial for NAFLD patients.

2. Objectives

Examination of the ultrasound measurement of fatty liver is important during the follow-up of patients treated for chronic HCV or NAFLD. Fatty liver is a common complication of the multi-metabolic syndrome, chronic viral hepatitis, and other chronic liver diseases. US measurement of fatty liver can trigger a liver biopsy in many cases, and therefore significantly facilitates the diagnosis and follow-up of chronic liver diseases.

The US measurement of fatty liver helps to detect the fat content of circumscribed liver changes, as well as focal sparing and focal deposition.

Our aim was to investigate the use of ultrasound techniques with which we can measure fatty liver with similar accuracy to other techniques (CT, PET-CT, or abdominal MRI). We also would like to investigate the technical and biological factors affecting the accuracy and reproducibility of US fatty liver measurement. Our aim was to follow up on the selected patient groups with the measurement of fatty liver.

3. Methods

Methods used in the research study on the use of shear wave elastography in chronic hepatitis C virus (HCV) infection. The study included 35 patients diagnosed with chronic HCV infection who had achieved sustained virological response (SVR) after antiviral therapy. The patients were treated and followed up by the Department of Internal Medicine. The inclusion criteria were the patients had to be above 18 years of age, have anti-HCV antibody and HCV RNA positivity, and have attained SVR after antiviral therapy. Patients with

extrahepatic cholestasis, congestive heart failure, HCC, BMI above 30 kg/m2, pregnancy, breastfeeding, or unsuccessful SWE measurements were excluded.

The study used a Samsung RS85 Prestige US scanner equipped with the CA1-7A convex probe and S-ShearwaveTM application to perform shear wave elastography measurements. The examination was considered successful if the interquartile range of the individual measurement was less than 30% of the median, and at least 5 reliable LS values were collected.

Laboratory test results were also collected, such as serum creatinine, sodium, AST, ALT, GGT, albumin levels, platelet count, total bilirubin, and international normalization ratio (INR) from electronic medical records at baseline and after treatment. The FIB4, the MELD, and the Child-Turcotte-Pugh (CTP) scores were calculated using MDCalc, and patients were classified into low-risk and high-risk groups of esophageal varices needing treatment (VNT) based on Baveno VI guidelines.
Statistical analysis was performed using paired Student's-test and Fisher's exact test to compare LS values and laboratory parameters before and after the DAA therapy, and the odds ratios (OR) are reported with confidence intervals (CI) of 95%. Mean and standard deviation were reported for continuous variables, and frequency and percentage were reported for categorical variables.
Methods used to measure liver fat in patients was quantitative ultrasound examination using a Samsung RS85 Prestige ultrasound device. During this examination, the right lobe of the patient's liver is examined from an intercostal view during medium inhalation using a B-mode image. The tissue attenuation index (TAI) and tissue scattering index (TSI) measurements are performed using the quantitative imaging mode of the ultrasound equipment, with 5 pieces of ROI placed in the liver parenchyma based on the B-mode.

Five measurements are taken per patient, and the median value of the 5 measurements is used to measure liver fat. The distance between the liver and the skin surface is also measured in all cases. The second method described is magnetic resonance imaging (MRI) using a Philips Ingenia 1.5 T MRI device. During this examination, a multi-echo gradient echo sequence is prepared, and the proton density-based fat fraction of the liver is calculated using the MRQuantif software. The MRI-PDFF values are expressed as a percentage, which indicates the percentage of fat-bound protons compared to the total (water-bound and fat-bound) protons. Finally, the MRI-PDFF values are projected back onto the original MRI recording to create a color contour map that shows the heterogeneity of steatosis within the liver parenchyma. Patients were diagnosed with steatosis using a threshold of 5%, and severe steatosis was defined as an MRI-PDFF threshold of >10%.

The statistical analysis of the data included examining the normal distribution of continuous variables with the Shapiro-Wilk test, using the chi-square test for categorical variables and the Mann-Whitney U-test for continuous variables. The patients were classified into three groups based on the severity of steatosis, and the Kruskal-Wallis test and post hoc Dunn’s test were used to examine the difference in continuous variables between the groups. The Benjamini-Hochberg correction was performed on p-values due to multiple comparisons between groups. Spearman’s correlation analysis was used to examine the correlation of clinical parameters, and ROC curve analysis was used to evaluate the diagnostic accuracy of the TAI and TSI ultrasound methods. Univariate and multivariate linear regression analysis was performed to identify confounding factors affecting the accuracy of TAI and TSI measurements. The reproducibility of the measurements was examined with intraclass correlation coefficient (ICC) and Spearman's correlation analysis. RStudio and several program packages were used for statistical analysis.

4. Results

The results presented in the first study, it can be concluded that successful antiviral treatment significantly improved liver stiffness, as well as liver function, in patients with hepatitis C. The average liver elastography value of the patients decreased from 20.04 kPa before treatment to 11.34 kPa after treatment, which was statistically significant. The METAVIR score also showed notable improvement after the antiviral therapy, with a decrease in the number of cirrhotic patients. In addition, liver-specific enzymes and values improved significantly, with liver enzyme values decreasing and several markers returning to the normal range in correaltion with liver stiffness. The FIB4 score also indicated a significant improvement after the treatment were correalated. Overall, these findings suggest that successful antiviral treatment can improve liver health in patients with hepatitis C.

The study enrolled 101 participants with suspected fatty liver disease. Among the participants, 54 (53.5%) had hepatic steatosis, and 62 (61.4%) were clinically suspected to have NAFLD without secondary etiology. The study found a significant correlation between the transient acoustic radiation force impulse (TAI) and MRI-PDFF values for the detection of hepatic steatosis. The mean TAI value of all patients with ≥5% and <10% MRI-PDFF was significantly higher than controls without steatosis, and a significantly higher TAI was detected in patients with ≥10% MRI-PDFF compared with other subjects in the hepatic steatosis group. The area under the receiver operating characteristic curve (AUC) of TAI for the detection of ≥5% and ≥10% MRI-PDFF were 0.89 and 0.93, respectively, with high sensitivity, specificity, NPV, PPV, and accuracy.

In 52 of the patients, the examination was repeated on the same day by a second

examiner who was independent of the first examiner. The study found that there was an excellent and significant correlation between the values measured by the two examiners for TAI (ρ=0.94; $p<0.001$). There was also a significant but only moderately strong correlation for TSI (ρ=0.57; $p<0.001$). The Bland-Altmann diagram was used to show the difference between the values measured by the two examiners. The average difference for TAI was 0.01 cm/dB/MHz, and for TSI it was 1.92, which represents a minimal difference between the examiners. The measurements did not exceed the threshold of acceptability (±1.96 standard deviation) for either TAI or TSI, indicating good reproducibility of the measurements. The study concluded that TAI is an effective tool for the noninvasive diagnosis of hepatic steatosis.

5. Conclusion

Overall, our research suggests that quantitative ultrasound measurement of liver fat content using TAI and TSI is a reliable method for detecting hepatic steatosis and diagnosing patients with NAFLD. In addition, TSI may also be useful for detecting NAFLD-associated liver fibrosis. Our other research recommends the addition of SWE to the follow-up imaging protocol to improve patient management and provide a more accurate assessment of fibrosis stage. The findings highlight the importance of using quantitative ultrasound methods in the evaluation of initial and follow-up ultrasound of patients with liver disease.

6. Bibliography Of The Candidate's Publications

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Cumulative impact factor of the candidate's publications related to the thesis: **∑IF: 5.169**

Total cumulative impact factor of the candidate's publications: **∑IF: 24,976**