Transient Elastography for the Detection of Hepatocellular Carcinoma in Viral C Liver Cirrhosis. Is there something else than Increased Liver Stiffness?

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INTRODUCTION

Early detection of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C virus (HCV) infection represents an emerging health problem. The growing incidence of HCV infection, together with continuous improvements in the clinical management of patients have led to an increasing prevalence of liver cirrhosis, which is the most important pathway towards developing HCC. These patients are the perfect candidates for HCC surveillance, having an annual incidence of HCC ranging between 4.1 and 7% [1].

The prognosis of patients with liver cirrhosis and HCC is strictly related to the liver residual function and tumour size, as stated and extensively validated by the BCLC criteria [2]; thus, non-invasive early detection of HCC is of major importance. Multiple parameters widely available in routine clinical practice were proposed for the detection of early HCC in patients with well-preserved liver function and various algorithms emerged for the diagnosis and follow-up of patients with HCV liver cirrhosis [3, 4]. Some of these models pointed out the individual role of increased liver stiffness (LS) measurements and serological markers as predictive biomarkers of HCC [5], but none of the studies evaluated the combined role of these main predictors.

Transient elastography (TE) is an imaging technique that measures LS under the assumption of pure elasticity of the
tissue [6]. Transient elastography accurately estimates and stages liver fibrosis, so that it is considered a surrogate for liver biopsy from this point of view. Transient elastography has been extensively validated over the last years, mainly in patients with chronic hepatitis C and it has been proved that its best accuracy is for detecting liver cirrhosis [7]. In the setting of liver cirrhosis, increased LS is associated with episodes of decompensation (high grade oesophageal varices - EV and/or bleeding, development of ascites) as well as with the presence of HCC [8], meaning that increased LS value alone cannot be an accurate predictor for HCC. The main drawbacks of TE are the technique difficulties in evaluating obese patients and the overestimation of liver fibrosis stage due to inflammation [9-13], central venous pressure [14], or cholestasis [15]. Transient elastography appears to be also influenced by the confounding effects resulting from attenuation and propagation of the acoustic waves in visco-elastic materials [11].

The aim of the present study was to study the performance of LS measurement data and of common biochemical parameters for the diagnosis of HCC in HCV related liver cirrhosis.

MATERIAL AND METHODS

Patients

A cohort of consecutive patients previously diagnosed with HCV liver cirrhosis (either biopsy proven, or having unequivocal clinical, biological and imaging features) was retrospectively included in the study according to following criteria: age between 18 and 80 years, positive anti-HCV antibodies for at least 6 months, positive HCV RNA and compensated disease (Child-Pugh A or B with no ascites). All patients were naïve for the antiviral treatment. We excluded from the study patients with other etiology of chronic liver diseases such as: hepatitis B or HIV co-infection, ethanol induced or autoimmune liver disease, Wilson’s disease, hemochromatosis or α1-antitripsin deficiency. They were matched by sex, age, BMI and years of HCV infection with another cohort of patients having early HCV patients having either liver cirrhosis alone or associated than 2,000 examinations performed at the time of the study performed by experienced operators (DF, ML), with more previous studies suggested [20, 21]. All LS measurements were number of validated LS measurements divided by the number of total measurements. Only examinations with a success rate of at least 60% were further analysed [12]. Since we wanted to fully investigate the influence of stiffness related parameters, the interquartile range (IQR) lower than 30% of the median value was not considered an exclusion criteria, as previous studies suggested [20, 21]. All LS measurements were performed by experienced operators (DF, ML), with more than 2,000 examinations performed at the time of the study patients with chronic liver diseases.

Liver stiffness measurement

Liver stiffness measurements were performed in the right liver lobe using one-dimension transient (impulsional) elastography (FibroScan®, Echosens, Paris, France) following the technical background and examination procedure as previously described [18]. Liver stiffness measurement was performed only after ultrasound guidance, in order to avoid the presence of focal liver lesions into the acquisition window. The medium probe was used for all patients. The results were expressed in kilopascals (kPa). The median value of 10 successful measurements was kept as a representative of the LS, according to the manufacturer’s recommendations and previous evidence [19]. The success rate was calculated as the number of validated LS measurements divided by the number of total measurements. Only examinations with a success rate of at least 60% were further analysed [12]. Since the p-values for all the variables included in the analysis. Patients in the HCC group were eventually excluded, if six months after their participation in the study, they exceeded the Milan criteria (either because of the size, or the number of the focal lesions) at a follow-up ultrasound evaluation. All patients also underwent oesophagoscopy for the assessment and grading of EV either during current admission, or in the previous 6 months interval. When present, EV were graded according to their size as follows: (i) grade 1: small, straight EV; (ii) grade 2: enlarged, tortuous EV occupying less than one third of the lumen; and (iii) grade 3: large, coil-shaped EV occupying more than one third of the lumen.

The study was designed in full accordance with the Declaration of Human Rights (Helsinki, 1975) and its further revisions and was previously approved by the Hospital Ethics Committee. All patients gave their written informed consent before enrolling into the study.

Statistical analysis

Mean LS values for the two groups were compared and introduced in a regression model of diagnosis. Data was analysed using MedCalc® 9.3.9.0. package for Windows. Data in text and tables are reported as mean (95%CI) or percentage (%). Demographic variables were assessed using descriptive statistics. Differences in mean values were tested using the t-test. The predictive value of biochemical and imaging parameters on HCC diagnosis was investigated through univariate and multivariate regression analysis. Variables that showed a significant relationship (p<0.05) with the presence of HCC in univariate analysis were included in multivariate regression analysis, in order to evaluate their simultaneous influence. We computed the model’s R-squared, the standardized and unstandardized weights, partial correlation coefficients and the p-values for all the variables included in the analysis. The relationship between different parameters and tumor size

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was tested through non-parametric Spearman correlation coefficients (r). The diagnostic performance of the LS and the regression model were assessed using the receiver operating characteristic (ROC) curves analysis. Optimal cut-off values were chosen by using a common optimization step that maximized the Youden index for predicting HCC. The sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios (LR) were computed from the same data, without further adjustments. A p value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Each cohort of the case-matching study consisted of 72 HCV cirrhotic patients, with or without HCC nodules. Another 40 HCV liver cirrhosis patients were included in the validation group. Clinical and biochemical characteristics of the study patients are summarized in Table I.

Liver stiffness values in the study population

In the case-matching group LS values ranged from 23 to 75 kPa, with a mean value of 27 kPa in patients without HCC, significantly lower than the mean LS value in those with HCC - 42 kPa (p<0.001), as shown in Fig. 1. Liver stiffness alone showed a poor performance for the diagnosis of HCC, as it results from the ROC curve analysis (Fig. 2A).

Regression analysis for HCC detection

In the univariate analysis ALAT, AP, GGT, platelets count, AFP, large oesophageal varices, LS and IQR were statistically significantly different in patients with HCC vs those without (Table I). In the multiple regression analysis, only four variables statistically correlated with the presence of HCC: LS, IQR, AFP and ALAT, of which LS had the highest odds ratio (OR) (Table II).

The four parameters together explained 64.5% of the variance of the HCC (R²=0.645, p<0.0001) and uniquely explained 45.45% of the amount of R², with LS making the largest unique contribution; the difference of 19.05% (64.5% - 45.45%) was accounted for by the joint contribution of the four parameters. Using the coefficients obtained in the regression analysis, the prediction model computed from this study can be expressed as follows:

Regression equation (LogitHCC)=1.92*LS+1.49*IQR+0.04*AFP+0.02*ALAT - 3.54

Analysing the performance of the predictive model for assessing the presence of HCC in the study population using the ROC curve method, we obtained a cut-off value of 0.5, a probability of 86% that a randomly selected individual from the HCC group has a test result indicating higher suspicion than that for a randomly chosen individual from the negative group (Fig. 2B, Table III).

We also analysed the relationship between the HCC size and each parameter included in the regression equation and we found a high positive correlation for LS (r=0.77, p<0.0001) and IQR (r=0.71, p<0.0001) and a moderate but significant correlation with the values of AFP (r=0.42, p=0.03).
Validation of the HCC model

In the validation group, the mean LS value was 37 kPa, ranging between 23 and 75 kPa.

Assessing the performance of the predictive model in the validation population and plotting the ROC curve for the prediction of HCC, using the same cut-off value, we still obtained a good AUROC and comparable Se, Sp, PPV and NPV figures (Fig. 3, Table III).

Table I. Baseline characteristics of the patients included in the study*

<table>
<thead>
<tr>
<th>Patients' characteristics</th>
<th>Case-matching group</th>
<th>Validation group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cirrhosis without HCC</td>
<td>Liver cirrhosis with HCC</td>
<td>p</td>
</tr>
<tr>
<td>Nr.</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Age</td>
<td>62.23 (59.21-66.25)</td>
<td>62.23 (59.21-66.25)</td>
</tr>
<tr>
<td>Male sex</td>
<td>48.61%</td>
<td>48.61%</td>
</tr>
<tr>
<td>BMI</td>
<td>27.43 (24.11 – 29.44)</td>
<td>27.21 (24.21 – 29.11)</td>
</tr>
<tr>
<td>ASAT (U/I)</td>
<td>89.26 (74.11-100.40)</td>
<td>92.26 (76.81-107.67)</td>
</tr>
<tr>
<td>ALAT (U/I)</td>
<td>77.38 (63.72-91.04)</td>
<td>98.36 (82.33-114.39)</td>
</tr>
<tr>
<td>AP (U/I)</td>
<td>270.07 (241.32-298.82)</td>
<td>358.72 (291.53-425.91)</td>
</tr>
<tr>
<td>GGT (U/I)</td>
<td>116.82 (81.05-152.59)</td>
<td>151.85 (110.34-193.36)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.98 (0.86-1.09)</td>
<td>1.73 (1.45-2.02)</td>
</tr>
<tr>
<td>Platelets (10³/mm³)</td>
<td>125.38 (110.84-39.91)</td>
<td>124.35 (96.88-151.82)</td>
</tr>
<tr>
<td>AFP (U/I)</td>
<td>16.38 (12.18-20.57)</td>
<td>119 (28.77-209.28)</td>
</tr>
</tbody>
</table>

* Data are expressed as absolute numbers, percentage or mean values.

Table II. Parameters independently associated with presence of HCC

<table>
<thead>
<tr>
<th>Main predictors</th>
<th>OR*</th>
<th>95%CI</th>
<th>Coefficient</th>
<th>Std. Error</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM</td>
<td>8.27</td>
<td>2.58 - 26.46</td>
<td>1.92</td>
<td>0.018</td>
<td>0.0001</td>
</tr>
<tr>
<td>IQR</td>
<td>1.16</td>
<td>1.04 - 1.29</td>
<td>1.49</td>
<td>0.055</td>
<td>0.0001</td>
</tr>
<tr>
<td>ALAT</td>
<td>1.01</td>
<td>0.96 - 0.99</td>
<td>0.02</td>
<td>0.009</td>
<td>0.004</td>
</tr>
<tr>
<td>AFP</td>
<td>1.04</td>
<td>1.01-1.08</td>
<td>0.04</td>
<td>0.01</td>
<td>0.009</td>
</tr>
</tbody>
</table>

OR – Odds Ratio, CI – confidence interval, Std. Error- Standard Error, LS - liver stiffness, IQR - Interquartile Range, ALAT - alanine aminotransferase, AFP - alpha-fetoprotein

Validation of the HCC model

In the validation group, the mean LS value was 37 kPa, ranging between 23 and 75 kPa.

Assessing the performance of the predictive model in the validation population and plotting the ROC curve for the prediction of HCC, using the same cut-off value, we still obtained a good AUROC and comparable Se, Sp, PPV and NPV figures (Fig. 3, Table III).

DISCUSSION

The present paper confirms the current knowledge that increased LS, AFP and serum transaminases are predictors of the presence of HCC in HCV liver cirrhosis patients. Besides
that, we demonstrated that increased IQR might be also a predictor for HCC, raising the question of a “stiffness shadow” generated by HCC into the cirrhotic liver. We also managed to compute a prognostic model based on regression analysis, that predicted the presence of HCC in both training and validation cohort with good accuracy.

Liver stiffness measurement using TE is recognised as accurately assessing the stage of liver fibrosis in patients with chronic HCV infection [12, 18], recorded values increasing as the liver disease progresses, and the highest LS being specific for cirrhotic patients with associated HCC [10]. Therefore, it was thought that LS measurement might be a valuable noninvasive tool for assessing the presence of, or the risk of developing liver cancer. The first studies reported a cut-off value of 53.7 kPa as suggestive for the presence of HCC in HCV cirrhotic patients [10], especially if total serum bilirubin was higher than 1.0 mg/dL [20]. However, the proposed threshold was not independently validated, and ranged between 12.5 and 53.7 kPa in other reports [5, 20, 21]. In Foucher’s study [10] the 53.7 kPa value was obtained from a subset of only 19 patients with HCC, and the 5% and 95% were 37% and 87%, respectively, with PPV and NPV of 30% and 90%. In addition, the study population in all mentioned papers was heterogeneous (HBV, HCV and ethanol), whereas our study included only HCV patients. For all the reasons we chose to determine our own cut-off value (38.5 kPa). Optimal cut-off values were chosen by using a common optimization step that maximized the Youden index and Se,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCC vs no HCC study group</th>
<th>HCC vs no HCC validation group</th>
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<tbody>
<tr>
<td>Se (%) (95%CI)</td>
<td>73.81 (58.86-1)</td>
<td>78.05 (62.89-1)</td>
</tr>
<tr>
<td>Sp (%) (95%CI)</td>
<td>96.15 (86.89-95)</td>
<td>69.70 (59.8-84.5)</td>
</tr>
<tr>
<td>+LR</td>
<td>34.63</td>
<td>28.63</td>
</tr>
<tr>
<td>-LR</td>
<td>0.27</td>
<td>0.31</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>97.18</td>
<td>72</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>78.07</td>
<td>76</td>
</tr>
<tr>
<td>AUROC (95%CI)</td>
<td>0.86 (0.76-0.92)</td>
<td>0.80 (0.65-0.89)</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Se - sensitivity, Sp - specificity, PPV - positive predictive value, NPV - negative predictive value, CI - confidence interval, +LR - Positive likelihood ratio, -LR - Negative likelihood ratio, AUROC - area under ROC curve, SE - standard error

Another important and surprising parameter was the IQR percentage of LS measurements. An IQR greater than 39% of median LS value resulted in being a good indicator and an important predictor of HCC presence. Interquartile range represents the interval including 50% of patients above and below the median. According to the manufacturer’s suggestion, the IQR/LS should be less than 30% of the median value [19]. Only a few studies investigated this issue, therefore the interpretation of results is derived more from personal experience and from the manufacturer’s advice. However, even lower values (<20%) [23] have been proposed in order to obtain a better concordance with liver biopsy. If gross technical operation errors are eliminated, than an IQR value greater than 30% indicates that structure disintegration is present in the liver. By finding a significantly higher IQR value (39.15% vs. 22.14%; p=0.001) in the HCC group, it means that this tissue unrest is due to the presence of HCC itself. It cannot be stated that HCC increases the LS because of its structure, as it is well known that liver cancer is a soft tumour [24]. More probably, it is the shear stress produced by the chaotic growth of the tumour inside of an already hard medium (the cirrhotic tissue) that induces the LS and the IQR increment. This finding supports the hypothesis of Mueller and Sandrin, according to which not only matrix but also pressure-associated conditions influence LS [25]. This inhomogeneous distribution of LS appears to generate a “stiffness shadow”, a finding that may have important clinical implications: if a certain patient with HCV cirrhosis, followed up by TE among other methods, suddenly develops an increase in LS median value, as well as in IQR, it may indicate the presence of HCC and may speed up the referral to other diagnostic techniques.

In our study, the presence of HCC was associated with high ALAT values, as previously reported [26], an association stronger if the ALAT increase is persistent [27]. Surprisingly, we found no correlation between large (grade 2 and 3) EV and the presence of HCC, although it is known that the two conditions are usually associated and each of them predict a poor outcome of the other [28]. As a common practice, AFP is widely used for HCC diagnosis, despite its low sensitivity and specificity [29]. However, high AFP levels at baseline were associated with an increased risk for HCC in HCV infected patients [30]. In our study, increased AFP was an independent predictor of HCC presence, but not an important variable in the regression model.

Our findings support the idea that each independent predictor, LS, IQR, ALAT and AFP, have a large role in the detection of HCC. The prognostic model is based on logistic regression, because this analysis finds the best fitting (yet biologically reasonable) model to describe the relationship between the outcome (HCC) and all independent variables. The four identified parameters predicted 64.5% of the HCC, with LS having the highest predictive power. The size and the direction of the relationship suggest that higher LS values are obtained for patients with HCC. The prognosis model using this formula had a good diagnostic performance, with an AUROC of 0.86 in the training set, and 0.8 in the validation one.
The efficacy of a predictor should be assessed under ideal conditions, which we tried to reproduce in a case-matching study by assuring a homogeneous “background noise” in the two groups [31]. By trying to generate the same baseline conditions, we also overcame some of the critical aspects of case-control studies: the selection of cases, recall bias, and surveillance bias. Therefore, we enrolled only patients under surveillance according to the latest recommendations [2, 32] and with the same frequency. Additionally, we matched the patients according to age, sex, BMI and history of HCV infection - because recent reports suggest a direct correlation between these parameters and the presence of HCC [33-36]. In this way, we could overcome the issue of BMI, which strongly determines the outcome of LS measurements [37].

To our knowledge, this is the first study that reports the combined role of LS measurement (median value and variance - IQR) and serum markers (AFP and ALAT) in the prediction of HCC in chronic HCV patients, with good performance. However, the most important finding of the study seems to be the increased IQR as a predictor of HCC presence. Since TE is a common examination in outpatient settings, the finding of an elevated LS value (e.g. >38 kPa, as our data suggest) as well as of an increased IQR during the follow-up of a patient with compensated liver cirrhosis may raise awareness for early HCC and may hasten the referral to confirmatory imaging techniques. This will not become a screening method, but will definitely improve the screening performance, by selecting the high risk patients for further confirmatory investigations.

CONCLUSION

In patients with HCV related cirrhosis, the presence of HCC is associated with increased LS and IQR values, as well as with high ALAT and AFP serum levels. Combining these four parameters into a model based on logistic regression analysis, liver cancer may be predicted with good accuracy. Stiffness changes, however, seem to be more complex, generating a “shadow” that might be characteristic for HCC, but this finding needs to be further investigated. Nevertheless, we believe that such an approach could be used to identify patients at high risk or, on the contrary, patients with no risk of HCC in order to limit the screening and decrease the number of useless procedures.

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Conflicts of interest: None to declare.

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