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Impact of Fibroscan® on management of chronic viral hepatitis in clinical practice

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ABSTRACT

Background. Liver stiffness measurement (LSM) using Fibroscan® is an increasingly popular non-invasive method for quantifying liver fibrosis in patients with chronic viral hepatitis. We aimed to explore potential impact of Fibroscan® on clinical management. **Material and methods.** 133 patients with chronic hepatitis B (HBV, n = 75) or C (HCV, n = 58) underwent Fibroscan® measurement. LSM results were compared with liver biopsy results, ultrasound, and APRI-scores, and the impact of LSM on clinical management was evaluated. **Results.** LSM results indicated fibrosis stage F0-F1 in 84 patients (63%), F2 in 28 (21%), F3 in 8 (6%), and F4 in 13 patients (10%). Nineteen patients had liver biopsies within one year of LSM. In ten patients, LSM and biopsy showed the same fibrosis stage, in 8 there was one stage difference, and in 1 three stages difference. Ultrasound only showed cirrhosis in three patients, who all exhibited advanced cirrhosis at LSM. There was a statistically significant, but weak correlation between LSM results and APRI scores (r = 0.31, p-value < 0.001). LSM results changed clinical management in 39% of patients (55 cases): in 15 patients antiviral treatment was indicated, in 21 patients surveillance for hepatocellular carcinoma was indicated, and 19 successfully treated hepatitis C patients could be discharged from clinical follow-up in absence of severe fibrosis or cirrhosis. **Conclusion.** LSM appears to be a valuable non-invasive tool to manage patients with chronic viral hepatitis in clinical practice.

Key words. Biopsy. Fibrosis. Hepatitis B. Hepatitis C. Liver cirrhosis. Ultrasonography.

INTRODUCTION

Hepatitis B and C are among the most important causes of liver cirrhosis worldwide. During the last decade, there have been dramatic developments in the treatment of viral hepatitis. Hepatitis C (HCV) can be cured with current standard-of-care treatment (pegylated interferon plus ribavirin) in about 50% of patients with virus genotypes 1 or 4, and in 80-90% of patients with genotypes 2 or 3.[1-3] Although the results of hepatitis B (HBV) treatment are less satisfying, significant progress has also been made in this field. To treat hepatitis B, one can choose between treatment with pegylated interferon (usually for one year)[4,5] or suppression of HBV DNA by long-term treatment with powerful nucleos(t)ide analogues like entecavir or tenofovir.[6-8]

The extent of liver fibrosis often plays an important role in the decision to start treatment in patients with viral hepatitis. Moreover, patients with viral hepatitis and severe liver fibrosis or cirrhosis should have ultrasonographic surveillance for hepatocellular carcinoma (HCC).[9] Liver biopsy still has a central role in the assessment of the extent of liver fibrosis. Disadvantages of liver biopsies are a (small) risk of complications and mortality, and the risk of underestimating the extent of fibrosis because of sampling error, depending on biopsy length.[10] A relatively new alternative for the assessment of liver fibrosis is liver stiffness measurement (LSM) using Fibroscan,® which determines the stiffness of the liver as a parameter of the extent of fibrosis or cirrhosis.[11,12] Another possibility is to assess the extent of fibrosis

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using a combination of serum markers, for example APRI (the AST to Platelet Ratio Index).[13]

Currently, Fibroscan® devices are not widely available, but their use is expected to increase. The reliability of liver stiffness measurements has been demonstrated in patients with viral hepatitis and with the cholestatic liver diseases primary biliary cirrhosis or primary sclerosing cholangitis.[14,15] An advantage of Fibroscan® is that measurements can easily be repeated, to monitor progression of fibrosis. We expect that physicians from various disciplines will be confronted with LSM with increasing frequency. Virtually no data are available on the impact of Fibroscan® on patient management in clinical practice. Fibroscan® could affect patient management by indicating need for antiviral therapy based on presence of significant fibrosis, need for hepatocellular cancer surveillance based on presence of severe fibrosis or cirrhosis or allowing patient discharge from further follow up after successful antiviral therapy based on absence of severe fibrosis. We therefore report our experiences with liver stiffness measurements using Fibroscan® in patient management in 133 patients with chronic viral hepatitis.

MATERIAL AND METHODS

Between 2006 and 2008, 142 patients with chronic hepatitis B or C from the University Medical Center Utrecht underwent liver stiffness measurements. In nine patients (6%) no reliable LSM results could be obtained. The remaining 133 patients were included in this study. Antiviral treatment was recommended after LSM based on AASL and EASL guidelines.[16-19] LSM results indicating significant fibrosis (≥ stage F2), in combination with significant viral load (> 2 x 10^3 IU/mL or > 2 x 10^4 IU/mL, depending on HBeAg status) signified a treatment indication for hepatitis B. In case of hepatitis C genotypes 2 or 3, antiviral treatment was advised regardless of LSM results. For genotypes 1 and 4, treatment was recommended in case of fibrosis stage ≥ F2. Surveillance for hepatocellular carcinoma was advised in case of fibrosis stage ≥ F3 (severe fibrosis or cirrhosis).[9] Hepatitis C patients who had sustained virological response after antiviral treatment and LSM results ≤ F2 could be discharged from clinical follow-up.

Liver stiffness measurements

Indication to perform Fibroscan® was need to obtain non-invasive information on extent of fibrosis in patients with chronic viral hepatitis. This information was either not present from other investigations or alternatively, served as baseline measurement for future follow up. Liver stiffness measurements were performed by one experienced investigator, who was not informed about clinical patient data, using a Fibroscan® device (Echosens, Paris, France). The Fibroscan® is a mobile device, which sends an elastic shear wave through the liver. The velocity of propagation of this wave is assessed as a measure of the elasticity, or the stiffness, of the liver tissue. Higher liver stiffness, indicating more fibrosis, causes the shear wave to move faster.[11] Liver stiffness is expressed in kilopascals (kPa). More information about Fibroscan® can be found at www.echosens.com. Before each liver stiffness measurement, ultrasound examination was performed to exclude ascites, vascular structures, cysts, and other abnormalities that could influence the reliability of the measurements. Liver stiffness measurements were performed on the right lobe of the liver, in an intercostal space, with the patient positioned on his back with the right arm in maximal abduction. Measurement depths were 25 to 65 mm below the skin surface. The median value of ten successful measurements was considered representative for the stiffness of the liver. Only results with at least ten successful measurements and a success percentage (defined as the number of successful measurements divided by the total number of measurements) of at least 60% were considered reliable and included in the analyses. Results were categorized according to the criteria by Castera, *et al.*:[20]

- < 7.1 kPa no or minimal fibrosis (F0-F1).
- 7.1-9.4 kPa moderate fibrosis (F2).
- 9.5-12.4 kPa severe fibrosis (F3).
- ≥ 12.5 kPa cirrhosis (F4).

Laboratory parameters and APRI score

Serum levels of AST, ALT, γ-GT, total bilirubin, alkaline phosphatase, albumin, platelet counts, and prothrombin times were measured by standard assays at our hospital laboratory, within 6 months' time of liver stiffness measurements. APRI scores were calculated for all patients as (AST/upper level of normal range for AST)/platelet count x 100. The APRI score can be used as a predictive measure for the extent of liver fibrosis.[21] Normal scores are between 0 and 0.5. Scores above 0.5 indicate significant fibrosis (≥ F2), while scores higher than 1 suggest cirrhosis (F4).[13,21]

Liver biopsies

In several patients liver biopsies had been performed as part of standard care. Decision to perform liver biopsy had been taken by the treating physician, based on clinical indications such as increased transaminase values, increased age or other risk factors for significant fibrosis. Fibrosis was scored by an experienced pathologist using the METAVIR classification.[22] Results of liver biopsies performed within one year of liver stiffness measurements were included in the analyses.

Ultrasound examinations

Abdominal ultrasound examinations evaluated by an experienced radiologist and performed within six months of liver stiffness measurements were used in this study.

Statistical analyses

For continuous variables medians, ranges and interquartile ranges were calculated. Categorical variables were reported as proportions. A statistically significant difference in LSM results between HBV and HCV patients was assumed when the 95% confidence intervals of the proportions of patients with a specific fibrosis stage in either group did not overlap. Spearman's correlation coefficient was used to assess the correlation between LSM results and APRI scores. A p-value < 0.05 was considered statistically significant. There is no absolute gold standard for staging liver fibrosis in clinical practice, since liver biopsies may underestimate degree of fibrosis due to sampling error. In the current work, sensitivity, specificity, positive and negative predictive values of LSM to predict significant fibrosis, severe fibrosis or cirrhosis with liver biopsies are given in those patients who had liver biopsy within 1 year from LSM measurement. We also calculated sensitivity, specificity and predictive values of abdominal ultrasoundation and APRI score to predict cirrhosis as measured with LSM. Data were analyzed using SPSS version 16.0 (SPSS Inc, Chicago IL, USA).

RESULTS

Baseline characteristics

Liver stiffness measurements were not successful in nine of 142 patients (6%), because of obesity (n = 3), or a success percentage below 60% (n = 6). Baseline characteristics and laboratory parameters of the remaining 133 patients are given in table 1. Seventy-five patients had chronic hepatitis B (30 of whom were on long-term antiviral treatment with nucleos(t)ide analogues at time of LSM), and 58 had chronic hepatitis C. Of the latter group, 37 patients had undergone antiviral treatment before LSM, with sustained virological response in 22 patients (59%). Twelve patients (9%) were co-infected with HIV.

Table 1. Baseline characteristics of 133 patients with chronic hepatitis B or C.

Total number of patients	133
Male gender	89 (67%)
Mean age in years (range)	41.2 (16-71)
Hepatitis B	75 (56%)
Antiviral treatment	30 (40%)
Hepatitis C	58 (44%)
Treatment naïve	21 (36%)
Previous unsuccessful antiviral treatment	15 (26%)
Previous successful antiviral treatment	22 (38%)
HIV co-infection	12 (9%)
BMI (kg/m^2)	25.2 (22.5-27.5)
AST (U/L)	32 (28-63)
ALT (U/L)	38 (23-47)
γ-GT (U/L)	29 (17-49)
Total bilirubin (μmol/L)	10 (6-15)
Alkaline phosphatase (U/L)	68 (58-80)
Albumin (g/L)	41 (39-44)
Platelet count (x10^9/L)	218 (171-252)
Prothrombin time (seconds)	13.9 (13.2-14.4)

Values are medians (interquartile range) or numbers (proportion). **LSM:** Liver stiffness measurement. **AST:** Aspartate aminotransferase. **ALT:** Alanine aminotransferase. **γ-GT:** γ-glutamyl-transpeptidase.

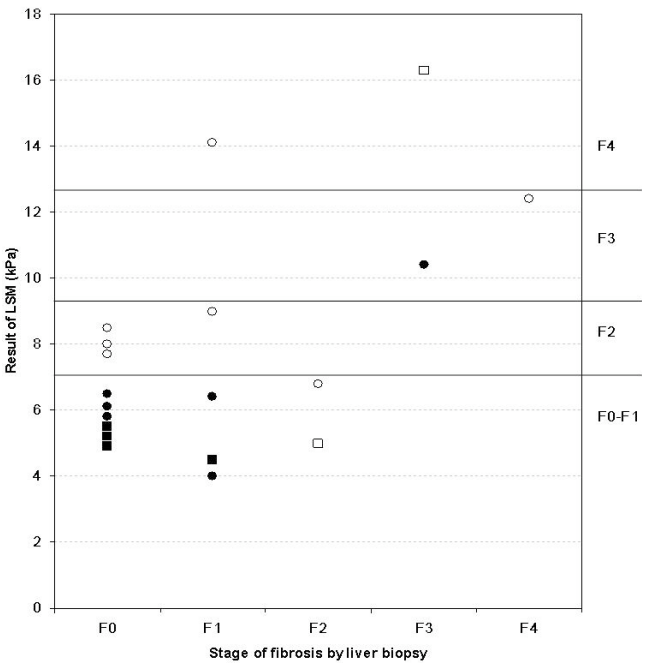
Liver stiffness measurements, liver biopsies, APRI scores, and ultrasound examinations

Table 2 shows LSM results for the total group and according to type of viral hepatitis. Eighty-four patients (63%) had no to minimal fibrosis (F0-F1) on LSM, 28 patients (21%) had moderate fibrosis (F2), 8 patients (6%) had severe fibrosis (F3), and 13 patients (10%) had cirrhosis (F4). No significant differences were seen between HBV and HCV patients. Fourteen patients had two successful liver stiffness measurements, with a median interval of 21 months (range 5-30 months). In 11 patients (79%) the results of the first and second measurements indicated the same fibrosis stage (F0-F1 in 10 patients and F4 in one patient). One HBV patient went from stage F0-F1 (5.7 kPa) to stage F3

Table 2. LSM results for the total group and according to type of hepatitis virus.

LSM result *	Total group, n = 133 (%)	HBV patients, n = 75 (%)	HCV patients, n = 58 (%)
Stage F0-F1	84 (63)	48 (64)	36 (62)
Stage F2	28 (21)	15 (20)	13 (22)
Stage F3	8 (6)	8 (11)	0
Stage F4	13 (10)	4 (5)	9 (16)

*LSM result classified according to criteria by Castera, *et al.*: F0-F1 < 7.1 kPa, F2 7.1-9.4 kPa, F3 9.5-12.4 kPa, and F4 ≥ 12.5 kPa.[20] LSM: Liver stiffness measurement. **HBV:** Hepatitis B virus. **HCV:** Hepatitis C virus.



- ● *Hepatitis B patients in whom LSM and biopsy show the same fibrosis stage (n = 6).*
- ○ *Hepatitis B patients in whom LSM and biopsy show different fibrosis stages (n = 7).*
- ■ *Hepatitis C patients in whom LSM and biopsy show the same fibrosis stage (n = 4).*
- □ *Hepatitis C patients in whom LSM and biopsy show different fibrosis stages (n = 2).*

***Figure 1.** Association between liver stiffness measurements (LSM) and liver biopsies.*

(10.2 kPa) in 8 months. Two HCV patients went from stage F2 (7.1 and 8.7 kPa, respectively) to stage F0-F1 (5.4 and 6.7 kPa) in 30 and 29 months, respectively. Both had relatively small absolute decreases in LSM results (1.7 and 2.0 kPa), and both underwent unsuccessful treatment with pegylated interferon and ribavirin before the first LSM.

In figure 1, liver biopsy results are compared with LSM results for the 19 patients in whom these investigations were performed within one year of each other. LSM and liver biopsy showed the same stage of liver fibrosis in 10 patients (53%), while LSM results were one fibrosis stage higher than biopsy results in 5 patients, three stages higher in one patient, and one stage lower in three patients. In only three patients the difference in kPa between their LSM results and the nearest limit of their liver biopsy fibrosis stage was > 2 kPa. Discrepancies between LSM and biopsy results were not related to ALT levels or biopsy lengths. Sensitivity, specificity, positive and negative predictive values of LSM to predict significant fibrosis (F0-F1 *vs.* ≥ F2) on liver biosy were 0.60, 0.64, 0.38 and 0.82, respectively, and to predict severe fibrosis (F0-F2 *vs.* ≥ F3) 1.0, 0.94, 0.75 and 1.0, respectively. Since numbers are limited, these data should be regarded with caution. Nevertheless, they are in line with previous reports, and suggest that Fibroscan® is more reliable to predict severe fibrosis or cirrhosis with indication for hepatocellular carcinoma surveillance than to predict significant fibrosis with indication for antiviral therapy.

In 65 patients (49%) abdominal ultrasound examinations were performed within six months of LSM. In three patients, all with LSM result stage F4 (13.9, 21.8 and 54.8 kPa, respectively), ultrasound results indicated liver cirrhosis. Further investigation showed thrombocytopenia in the patient with LSM result 21.8 kPa, and both thrombocytopenia and esophageal varices in the patient with LSM result 54.8 kPa. Both these patients had APRI-scores higher than 1.0 (1.04 and 1.33, respectively), while in the third patient the APRI-score was 0.46. In 13 of the remaining 62 patients LSM results indicated severe fibrosis (n = 3) or cirrhosis (n = 9) without any abnormalities at abdominal ultrasound investigation. Sensitivity, specificity, positive and negative predictive values of ultrasound to predict cirrhosis on Fibroscan® (F0-F3 *vs.* F4) were 0.25, 1.0, 1.0 and 0.85, respectively. Similar results were obtained for prediction of severe fibrosis (F0-F2 *vs.* ≥ F3: results not shown). These data suggest that

ultrasound is quite specific but not very sensitive to predict cirrhosis.

For 127 patients (95%) APRI scores could be calculated within six months of liver stiffness measurements (Table 3). The six patients for whom no APRI scores were available all had LSM result F0-F1. Median APRI score was 0.41 (range 0.14-3.51). APRI scores were 0-0.49 in 82 patients (65%), 0.50-0.99 in 33 patients (26%), and ≥ 1.00 in 12 patients (9%). Correlation between LSM results and APRI scores was statistically significant, but not very strong (Spearman's correlation coefficient 0.31, p-value < 0.001). Strong discrepancies were seen in 11 patients with LSM results F3 or F4 and APRI scores below 0.50, and in 6 patients with LSM results F0-F1 or F2 and APRI scores ≥ 1.00. Sensitivity, specificity, positive and negative predictive values of APRI score to predict significant fibrosis (F0-F1 *vs.* ≥ F2) on Fibroscan® were all below 0.5, and to predict cirrhosis (F0-F3 *vs.* F4) 0.38, 0.94, 0.42 and 0.93, respectively. Similar results were obtained for prediction of severe fibrosis (F0-F2 *vs.* ≥ F3: results

Table 3. Comparison of LSM results and APRI scores in 127 patients with chronic viral hepatitis.

APRI score	LSM result			
	F0-F1, n = 78 (%)	F2, n = 28 (%)	F3, n = 8 (%)	F4, n = 13 (%)
0-0.49	59 (76)	12 (43)	5 (63)	6 (46)
0.50-0.99	17 (22)	12 (43)	2 (25)	2 (15)
≥ 1.00	2 (2)	4 (14)	1 (12)	5 (39)

APRI: AST to platelet ratio index. **LSM:** Liver stiffness measurement.

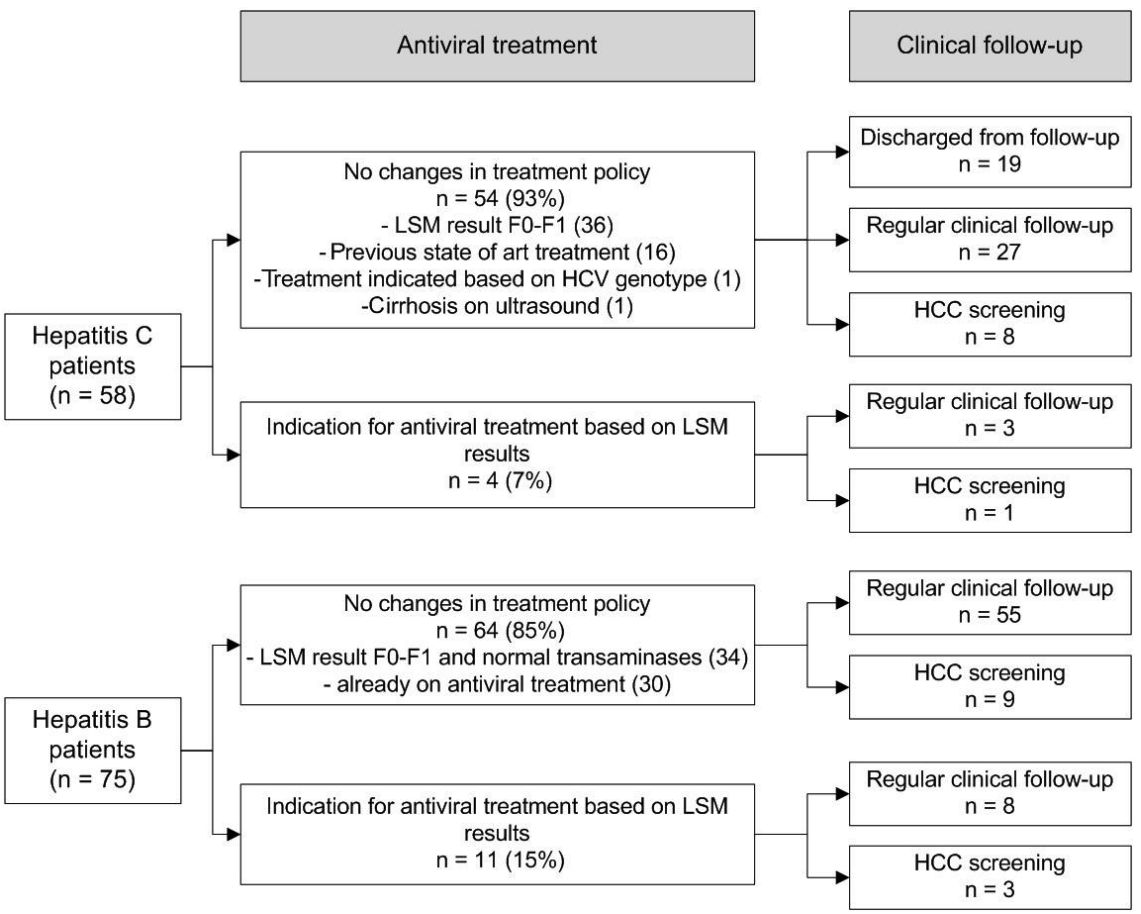


Figure 2. *Impact of liver stiffness measurements (LSM) with Fibroscan® on clinical management in 133 patients with chronic viral hepatitis.* ***HCC:*** *Hepatocellular carcinoma.*

not shown). These data suggest that APRI score could be of some help to indicate low probability of cirrhosis, but is of no value to predict moderate fibrosis.

Consequences of liver stiffness measurements for clinical practice

Figure 2 summarizes the impact of liver stiffness measurement on clinical practice. LSM results did not change decisions regarding antiviral treatment in 54 of 58 HCV patients (93%). In these patients LSM results were F0-F1, they had already undergone state of the art HCV treatment, antiviral treatment was already indicated based on a favorable HCV genotype, or abdominal ultrasound examination had already shown cirrhosis. Of the four remaining HCV patients, three had LSM result F2 (all HCV genotype 1 and previously untreated) and one F4 (genotype 1, previous unsuccessful pegylated interferon monotherapy), indicating need for antiviral treatment with pegylated interferon and ribavirin.

Thirty HBV patients were already on long-term antiviral treatment at time of LSM. Of the 45 treatment naive patients, 34 had LSM results F0-F1 and no indication for treatment based on normal transaminase levels. Of the remaining 11 patients, 8 had LSM result F2, one F3, and two F4, all with significant viral loads, indicating need for antiviral therapy. One additional HBV patient, who had two liver stiffness measurements in 8 months, and an increase in LSM results of 4.5 kPa during this period, started entecavir treatment after his second LSM.

In 21 patients (16%) HCC surveillance was initiated based on the presence of previously unknown severe fibrosis (n = 8) or cirrhosis (n = 13) on their LSM results.

Nineteen HCV patients could be discharged from further follow-up, because they underwent successful antiviral treatment and had LSM results ≤ F2, without previously known fibrosis stage.

DISCUSSION

Determining the extent of liver fibrosis provides important prognostic information in patients with chronic liver disease. Moreover, in viral hepatitis the extent of liver damage often is an important reason to either start or postpone antiviral treatment. Patients with viral hepatitis who have severe fibrosis or cirrhosis should also have surveillance for hepatocellular carcinoma.[9] Liver biopsies still have a central role in the assessment of the extent of liver fibrosis. They also provide information about inflammation severity and, occasionally, unexpected diagnoses can be made. Disadvantages of liver biopsies are complication risks and the risk of sampling error with underestimation of fibrosis.[10,23,24] Moreover, it is not feasible to frequently repeat liver biopsies, while the extent of liver damage can increase at relatively fast rates, especially in hepatitis B. In this paper we discuss our experience in clinical practice with liver stiffness measurements using Fibroscan® as a non-invasive alternative for liver biopsies in 133 patients with chronic viral hepatitis.

LSM results indicated no or minimal fibrosis (F0-F1) in 84 patients (63%), and cirrhosis (F4) in 13 patients (10%). In 19 patients liver biopsies were performed within six months of LSM. Our comparison of LSM with biopsies could be biased: liver biopsies could have been performed in those patients because treating physicians did not trust the LSM results, or the other way around. In 9 patients liver biopsies were performed before LSM, and in 10 patients after LSM. In 10 patients LSM and biopsy showed corresponding results, while in 8 other patients only one fibrosis stage difference was seen (often with very small differences in kPa between LSM result and the nearest limit of the biopsy fibrosis stage). Increased ALT levels have been reported to reduce the reliability of LSM, although results in this area are conflicting.[12,20,25-27] ALT levels did not appear to be associated with the discrepancies between LSM and biopsy results in our patients. However, in only a few patients ALT levels were increased above 100 U/L. Variation in biopsy lengths could not explain discrepancies between LSM and biopsies either. Whether such discrepancies are a result of limitations of LSM or of liver biopsy remains to be determined. Our data on sensitivity, specificity, and positive or negative predicitive value are in line with previous reports, and suggest that Fibroscan® is more reliable to predict severe fibrosis with indication for hepatocellular carcinoma surveillance than to predict significant fibrosis with indication for antiviral therapy. Nevertheless, our patient numbers are limited, and these data should be viewed with caution.

In 65 patients abdominal ultrasound examinations were performed within six months of LSM. Ultrasound was found to be highly specific, but not very sensitive to predict cirrhosis. As a consequence, no further investigations are necessary when cirrhosis is found on ultrasound, but further investigations may be considered if ultrasound is normal, depending on clinical judgement.

APRI scores are easy to calculate, and showed a significant, but not very strong correlation with LSM results ($r = 0.31$, p-value < 0.001), with large discrepancies between LSM results and APRI scores in 17 patients (13%). In our patients, APRI score was not a good parameter for significant fibrosis, but could be of some value to indicate low probability of cirrhosis.

Liver stiffness measurements had consequences for treatment policies in 39% of patients. In 15 untreated patients antiviral treatment was indicated based on increased LSM results, and HCC surveillance was indicated in 21 patients. Nineteen other patients with sustained virological response after treatment for HCV could be discharged from further follow-up based on their favorable LSM results.

A limitation of our study is the relatively low number of patients, especially for the comparison of LSM results with liver biopsy results. However, our study population will probably be representative for patient populations in many clinical practices worldwide, and our experience could aid other clinicians who are dealing with similar situations. Larger, prospective studies will have to clarify the use of both single and longitudinal LSM to assess the extent and progression of fibrosis in clinical practice.

CONCLUSION

Based on the experiences described in this paper, we conclude that liver stiffness measurement using Fibroscan® appears to be a valuable non-invasive addition to clinical practice in patients with chronic viral hepatitis.

ABBREVIATIONS

- **HCV:** Hepatitis C virus.
- **HBV:** Hepatitis B virus.
- **ULN:** Upper limit of normal.
- **HCC:** Hepatocellular carcinoma.
- **LSM:** Liver stiffness measurement.
- **APRI:** AST to Platelet Ratio Index.
- **kPa:** Kilopascal.
- **AST:** Aspartate aminotransferase.
- **ALT:** Alanine aminotransferase.
- **γ-GT:** γ-glutamyl-transpeptidase.
- **HIV:** Human immunodeficiency virus.

REFERENCES

1. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975-82.
2. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; 140: 346-55.
3. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet* 2001; 358: 958-65.
4. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomized trial. *Lancet* 2005; 365: 123-9.
5. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; 352: 2682-95.
6. Baldick CJ, Tenney DJ, Mazzucco CE, Eggers BJ, Rose RE, Pokornowski KA, Yu CF, et al. Comprehensive evaluation of hepatitis B virus reverse transcriptase substitutions associated with entecavir resistance. *Hepatology* 2008; 47: 1473-82.
7. Gish RG, Lok AS, Chang TT, de Man RA, Gadano A, Sollano J, Han KH, et al. Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. *Gastroenterology* 2007; 133: 1437-44.
8. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, Germanidis G, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. *N Engl J Med* 2008; 359: 2442-55.
9. Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; 42: 1208-36.
10. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; 38: 1449-57.
11. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; 29: 1705-13.
12. Ziol M, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, De Ledinghen V, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; 41: 48-54.
13. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38: 518-26.
14. Corpechot C, El Naggar A, Poujol-Robert A, Ziol M, Wendum D, Chazouilleres O, De Ledinghen V, et al. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006; 43: 1118-24.
15. Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; 134: 960-74.
16. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; 50: 227-42.
17. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49: 1335-74.
18. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; 50: 661-2.

19. Craxi A. EASL Clinical Practice Guidelines: Management of hepatitis C virus infection. *J Hepatol* 2011 [Epub ahead of print 28 February 2011].
20. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; 128: 343-50.
21. Shaheen AA, Myers RP. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis C-related fibrosis: a systematic review. *Hepatology* 2007; 46: 912-21.
22. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; 24: 289-93.
23. Manning DS, Afdhal NH. Diagnosis and quantitation of fibrosis. *Gastroenterology* 2008; 134: 1670-81.
24. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; 97: 2614-8.
25. Arena U, Vizzutti F, Abraldes JG, Corti G, Stasi C, Moscarella S, Milani S, et al. Reliability of transient elastography for the diagnosis of advanced fibrosis in chronic hepatitis C. *Gut* 2008; 57: 1288-93.
26. Coco B, Oliveri F, Maina AM, Ciccorossi P, Sacco R, Colombatto P, Bonino F, et al. Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. *J Viral Hepat* 2007; 14: 360-9.
27. Lupsor M, Badea R, Stefanescu H, Grigorescu M, Sparchez Z, Serban A, Branda H, et al. Analysis of histopathological changes that influence liver stiffness in chronic hepatitis C. Results from a cohort of 324 patients. *J Gastrointestin Liver Dis* 2008; 17: 155-63.