Liver International ISSN 1478-3223

CLINICAL STUDIES

Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B

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Keywords
chronic hepatitis B – fibrosis – liver biopsy – liver stiffness – transient elastography

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Received 6 February 2008
Accepted 7 April 2008

DOI:10.1111/j.1478-3231.2008.01802.x

Abstract

Background: The need for new non-invasive tools to assess liver fibrosis in chronic liver diseases has been largely advocated. Liver stiffness measurement (LSM) using transient elastography (FibroScan®, Echosens™) has been shown to be correlated to liver fibrosis in various chronic liver diseases. This study aims to assess its diagnosis accuracy in patients with chronic hepatitis B. *Patients and methods:* We prospectively enrolled 202 patients with chronic hepatitis B in a multicentre study. Patients underwent liver biopsy (LB) and LSM. METAVIR and Ishak liver fibrosis stages were assessed by two pathologists. *Results:* LSM or LB was considered unreliable in 29 patients. Statistical analysis was conducted in 173 patients. LSM was significantly ($P < 0.001$) correlated with METAVIR ($r = 0.65$) and Ishak fibrosis stage (0.65). The area under receiver-operating characteristic curves were 0.81 (95% confidence intervals, 0.73–0.86) for $F \geq 2$, 0.93 (0.88–0.96) for $F \geq 3$ and 0.93 (0.82–0.98) for $F = 4$. Optimal LSM cut-off values were 7.2 and 11.0 kPa for $F \geq 2$ and $F = 4$, respectively, by maximizing the sum D of sensitivity and specificity, and 7.2 and 18.2 kPa by maximizing the diagnosis accuracy. *Conclusion:* In conclusion, LSM appears to be reliable for detection of significant fibrosis or cirrhosis in HBV patients and cut-off values are only slightly different from those observed in HCV patients.

The prognosis and management of chronic liver diseases depend strongly on the degree of liver fibrosis. This is particularly true of hepatitis B virus (HBV)-related chronic hepatitis. Until recently, liver biopsy (LB) examination was the only way of evaluating liver fibrosis (1). However, LB examination is invasive and painful (2), can have life-threatening complications and is costly. The poor acceptability of LB examination can lead to treatment delays (3), and LB examination is difficult to repeat in generally asymptomatic patients. The accuracy of LB examination for assessing fibrosis has also been questioned because of sampling errors and intra- and interobserver variability that may lead to over- or understaging of fibrosis (4–6). There is thus a need for accurate non-invasive methods of measuring the degree of liver fibrosis. Proposed approaches include physical examination, ultrasound imaging (7, 8), routine biochemical and haematological tests (9–11) and surrogate serum fibrosis markers. Fibrosis scores based on combinations of several blood tests have been elaborated (12, 13). However, their accuracy is limited, especially for differentiating moderate and severe fibrosis. In addition, some conditions (technical or patient related) influencing blood tests may induce under- or overestimation of the fibrosis stage.

Previous studies have shown that liver stiffness measurement (LSM) accurately predicts hepatic fibrosis stage in patients with chronic hepatitis C (14–16), in patients with human immunodeficiency virus (HIV)–hepatitis C virus (HCV) coinfection (17) and in patients with chronic cholestatic liver disease such as primary biliary cirrhosis or primary sclerosing cholangitis (18). In particular, it has been shown that this technique has a high predictive value for the presence of cirrhosis in patients with chronic liver diseases (19, 20). However, the accuracy of this method to predict fibrosis stage in patients with chronic hepatitis B has not been specifically studied.

The objective of this prospective study was to assess the predictive value of LSM for liver fibrosis stage by comparing, in a cohort of patients with chronic hepatitis B, LSM and histological staging serving as a reference.

Patients and methods

Patients

Two hundred and two consecutive patients with chronic hepatitis B admitted for LB were enrolled in this study in five different French hospitals: Jean Verdier (Bondy), Henri Mondor (Créteil), Beaujon (Clichy), Saint Antoine (Paris) and Haut Lévêque (Pessac). Inclusion criteria were the presence of hepatitis B surface antigen, serum HBV-DNA levels

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$> 10^5$ copies/ml and liver histology compatible with chronic hepatitis. Patients with chronic alcohol intake or HCV coinfection and patients with ascites were excluded from the study. LSM was performed within 3 months of the LB. The protocol was in accordance with the Helsinki Declaration and was approved by an independent ethics committee. Patients fulfilling these criteria were enrolled after providing their written and informed consent. Blood parameters were evaluated on the same day that LSM was performed.

Transient elastography principle

Liver stiffness measurement was performed with Fibroscan® (EchoSens™, Paris, France), a medical device based on elastometry (or one-dimensional transient elastography). Details of the technical description and examination procedure have been described previously (21). Briefly, this system is equipped with a probe including an ultrasonic transducer mounted on the axis of a vibrator. A vibration of mild amplitude and low frequency is transmitted from the vibrator towards the tissue by the transducer itself. This vibration induces an elastic shear wave that propagates through the tissue. In the meantime, pulse-echo ultrasound acquisitions are performed to follow the propagation of the shear wave and measure its velocity, which is directly related to tissue stiffness (or elastic modulus). Results are expressed in kilopascal. The harder the tissue, the faster the shear wave propagation.

Liver stiffness measurements

Measurements were performed in the right lobe of the liver through the intercostal spaces on patients lying in the dorsal decubitus position with the right arm in maximal abduction. The tip of the probe transducer was covered with coupling gel and placed on the skin between the ribs at the level of the right lobe of the liver. The operator, assisted by ultrasound time-motion and A-mode images provided by the system, located a portion of the liver that was at least 6 cm thick and free of large vascular structures. Once the area of measurement had been located, the operator pressed the probe button to begin an acquisition. The measurement depth was between 25 and 65 mm. Several successful acquisitions were performed on each patient. The success rate was calculated as the ratio of the number of successful acquisitions over the total number of acquisitions. The median value was kept as representative of the liver elastic modulus. The entire examination lasted < 5 min. Only results of LSM obtained with at least seven successful acquisitions and a success rate of at least 50% were considered reliable.

Liver histology and quantification of liver fibrosis

Liver biopsies were fixed in formalin and paraffin embedded. Four-micrometre-thick sections were stained with haematoxylin–eosin–safran and picrosirius red. All biopsy specimens were analyzed by two experienced pathologists (M. Z. and P. B.) blinded to the results of LSM and clinical data. Liver biopsies that contained < 10 portal tracts (except for cirrhosis) were excluded from the histological analysis. Liver fibrosis and necroinflammatory activity were evaluated semiquantitatively according to the METAVIR scoring system (22) and fibrosis was also staged according to the Ishak scoring system (23). The fibrosis stage was assessed independently on each histological section by both pathologists. Thereafter, in case of discrepancies, histological sections were simultaneously reviewed using a multipipe microscope in order to reach a consensus. Activity was graded as A0, none; A1, mild; A2, moderate; and A3, severe. Steatosis was categorized by visual assessment as 0, none; 1, steatosis in 1–10% of hepatocytes; 2, in 10–30%; and 3, 30–100% of hepatocytes. The length of each LB specimen was also established in millimetres.

Statistical analysis

Agreement between the two pathologists who analyzed the histological sections was evaluated using the quadratic-weighted κ coefficient of Cohen. LSM do not follow a normal distribution; hence, univariate and multivariate analyzes were performed with the log transform of stiffness values. Univariate analysis was performed using Pearson's correlation coefficient. Multivariate analysis was performed using multiple regression of the log transform of liver stiffness vs the parameters that were significantly correlated in the univariate analysis. Gender was introduced in the multiple regression as a categorical variable and all the other parameters as continuous variables. The receiver-operating characteristic (ROC) curves were computed and areas under the curves as well as 95% confidence intervals (CI) were calculated with the Mann–Whitney statistic. Optimal cut-off values were defined using two different criteria: maximizing the sum of sensitivity and specificity or maximizing the diagnosis accuracy (percentage of patients diagnosed correctly). For each optimal cut-off value, the sensitivity, specificity, positive and negative predictive values, likelihood ratio and diagnosis accuracy were computed. All tests were two sided, with a significance level of 5%. Statistical analyzes were performed with NCSS 2004 (Statistical Systems, Kayville, UT, USA).

Results

Patients

Among the 202 included patients (Jean Verdier, 71; Henri Mondor, 48; Beaujon, 44; Saint Antoine, 20; and Haut Lévêque, 19), 15 (7.4%) had a non-interpretable LB and 14 (6.9%) had an LSM considered as non-reliable [out of these 14 patients, nine had a body mass index (BMI) above 25 kg/m^2]. The statistical analysis was therefore conducted on 173 patients, among whom eight had daily alcohol intake ≥ 40 g, two had hepatitis D virus coinfection and 11 had HIV coinfection. Table 1 summarizes the general characteristics of the patients included. Most (93%) of the 173 patients included in the statistical analysis had LB and LSM within the same day or the day after (mean delay 2 ± 9 days), and the mean ($\pm$ standard deviation) acquisition success rate was 90 ± 14%. The median interquartile range (IQR)/LSM ratio was 18% (IQR, 12–25%).

Histology

In the studied population, the mean biopsy length was 16 ± 6 (3–32) mm. Patient distribution for METAVIR and Ishak fibrosis stage, METAVIR activity grade and steatosis are presented in Tables 2 and 3 respectively. Pathologists were initially in agreement for 134 (77.5%) of the 173 liver biopsies analyzed using the METAVIR fibrosis stage (κ coefficient, 0.88) and for 113 (65.3%) using the Ishak fibrosis stage (κ coefficient, 0.87).

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Table 1. Characteristics of included patients

Men	115 (66.5%)
Mean age (years)	40.1 ± 12.8
Mean body mass index (kg/m^2)	24.5 ± 4.0
Median platelets (10^3/mm^3)	207 (156–235)
Median albumin (g/L)	44.5 (42.0–47.4)
Median prothrombin time (% of normal)	90 (81–98)
Median total bilirubin (μM/L)	11.0 (8.0–14.0)
Median γ-glutamyl transpeptidase (IU/L)	33 (20–70)
Median γ-globulin (g/L)	13.8 (11.0–16.7)
Median aspartate aminotransferase (IU/L)	35 (25–54)
Median alanine aminotransferase (IU/L)	54 (30–85)

Table 2. Patients' distribution for METAVIR and Ishak fibrosis stage

METAVIR	*N* (%)	Ishak	*N* (%)
0	16 (9.2)	0	14 (8.1)
1	70 (40.5)	1	41 (23.7)
2	44 (25.4)	2	39 (22.5)
3	29 (16.8)	3	34 (19.7)
4	14 (8.1)	4	17 (9.8)
		5	14 (8.1)
		6	14 (8.1)

Table 3. Patients' distribution for METAVIR activity grade and steatosis

Activity	*N* (%)	Steatosis	*N* (%)
0	45 (26.0)	0	97 (56.1)
1	93 (53.8)	1	50 (28.9)
2	27 (15.6)	2	14 (8.1)
3	8 (4.6)	3	12 (6.9)

Relationship between liver stiffness and histological and biological parameters

Figure 1 shows the box plots of liver stiffness values vs METAVIR and Ishak fibrosis scores as well as activity and steatosis. The median value (minimum − maximum) of liver stiffness compared with consensus METAVIR fibrosis stage: F0, 5.1 (2.5–8.5) kPa; F1, 6.0 (2.7–35.3) kPa; F2, 7.0 (2.8–17.6) kPa; F3, 12.8 (5.9–45.1) kPa; and F4, 23.7 (6.4–59.3) kPa.

Table 4 shows the results of the univariate analysis of liver stiffness vs BMI, histological and biological parameters. LSM was significantly correlated with the METAVIR fibrosis stage and the Ishak fibrosis stage. It was also correlated with METAVIR activity grade, platelet count, serum albumin, prothrombin time, γ-glutamyl transpeptidase activity, serum γ-globulin, aspartate aminotransferase and alanine aminotransferase activity, but not with steatosis, BMI and total bilirubin. A multivariate analysis was performed on LSM vs all significant parameters, except Ishak fibrosis score, which was strongly correlated with METAVIR fibrosis stage ($r = 0.95$, $P < 0.001$). In this multivariate analysis, LSM was significantly correlated with METAVIR fibrosis stage F (partial correlation coefficient r_p, 0.443, $P < 0.001$) and platelet count (r_p, − 0.229, $P = 0.037$).

Receiver-operating characteristic analysis

Figure 2 shows the ROC curves determined for the whole population according to three different fibrosis stage thresholds: F0 and F1 patients vs F2, F3 and F4 patients ($F \geq 2$); F0, F1 and F2 patients vs F3 and F4 patients ($F \geq 3$); and F0, F1, F2 and F3 patients vs F4 patients ($F = 4$). The areas under the ROC curves (95% CI) were 0.81 (0.73–0.86) for $F \geq 2$, 0.93 (0.88–0.96) for $F \geq 3$ and 0.93 (0.82–0.98) for $F = 4$.

No significant difference was observed between smaller and larger LBs (keeping the same breakdown of the population according to fibrosis stage) for the areas under the ROC curves. For smaller and larger liver biopsies, the areas under the ROC curves (95% CI) were 0.78 (0.66–0.86) and 0.83 (0.74–0.90) for $F \geq 2$, 0.93 (0.86–0.97) and 0.93 (0.83–0.97) for $F \geq 3$, 0.97 (0.90–0.99) and 0.88 (0.63–0.97) for $F = 4$ respectively.

No significant difference was observed between pathologist one and pathologist two for the areas under the ROC curves. For pathologist one and two, the areas under the ROC curves (95% CI) were 0.77 (0.69–0.83) and 0.81 (0.73–0.87) for $F \geq 2$, 0.93 (0.88–0.96) and 0.88 (0.81–0.93) for $F \geq 3$ and 0.92 (0.82–0.97) and 0.86 (0.71–0.94) for $F = 4$ respectively.

Table 5 gives the optimal cut-off values for the diagnosis of $F \geq 2$, $F \geq 3$ and $F = 4$ when choosing to maximize the sum of sensitivity and specificity or to maximize the diagnosis accuracy. The diagnosis accuracy was 76%, 90% and 94% for the diagnosis of fibrosis stage F2–F4, F3–F4 and F4 respectively. Four patients with LSM > 18.2 kPa had no cirrhosis: three patients had F3 fibrosis and one patient had F1 fibrosis. However, for the last patient, IQR/LSM was 87%.

Discussion

This prospective study shows that transient elastography is an efficient technique for the assessment of fibrosis in patients with chronic hepatitis B. LSM was well correlated with the histological METAVIR and Ishak scores, and discriminated well the patients with METAVIR F0–F1 vs F2–F4 (ROC curves 0.81, 0.73–0.86) and even better the patients with F0–F2 vs F3–F4 (ROC curves 0.93, 0.82–0.98).

This study shows that the performance of LSM in predicting liver fibrosis stage in patients with chronic hepatitis B is comparable to that observed in patients with chronic hepatitis C because in the study by Ziol *et al.* (16) and Castera *et al.* (14), the ROC curves were 0.82 for F0–F1 vs F2–F4 and 0.90 for F0–F2 vs F3–F4. LSM was very accurate for the diagnosis of bridging fibrosis or cirrhosis (90%). In clinical practice, such results could be of major relevance, mainly for excluding cirrhosis in patients with chronic hepatitis B. In the rare cirrhotic patients with LSM < 11 kPa, LSM results could be considered mainly as false negatives because macronodular cirrhosis is more common in chronic hepatitis B than in chronic hepatitis C. While the amount of fibrosis in micronodular cirrhosis is usually high and the diagnosis of cirrhosis correctly assessed in this case by LSM, this technique is not such an accurate tool for assessing liver architectural abnormalities with a limited amount of fibrosis such as in naïve patients with macronodular cirrhosis, characterized by large nodules delimited by thin septa. Similar discrepancies were observed in F3–F4 patients with chronic hepatitis C previously treated by interferon and/or with sustained virological response, a condition that could contribute to decreased liver fibrosis (20).

Likewise, in patients with chronic hepatitis C, LSM was less accurate in discriminating absence or mild fibrosis (F0 or F1) vs

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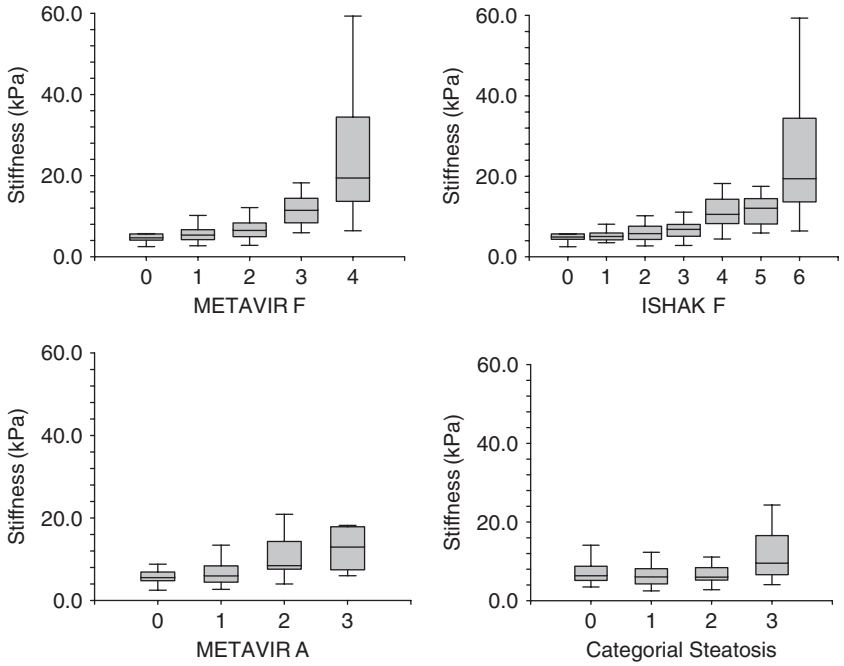

Fig. 1. Box plots of liver stiffness measurement vs METAVIR fibrosis stage, Ishak fibrosis stage, METAVIR activity grade and steatosis category.

Table 4. Pearson's correlation coefficient of the log transform of liver stiffness vs histological and biological parameters

Parameters	*r*	*P*
METAVIR fibrosis stage F	0.645	< 0.001
METAVIR activity grade A	0.358	< 0.001
Steatosis	0.008	0.916
Ishak fibrosis stage	0.652	< 0.001
Body mass index (kg/m^2)	0.007	0.929
Platelets (10^3/mm^3)	− 0.350	< 0.001
Albumin (g/L)	− 0.228	0.006
Prothrombin time (%)	− 0.224	0.004
Total bilirubin (μM/L)	0.091	0.249
γ-glutamyl transpeptidase (IU/L)	0.474	< 0.001
γ-globulin (g/L)	0.287	< 0.001
Aspartate aminotransferase (IU/L)	0.509	< 0.001
Alanine aminotransferase (IU/L)	0.348	< 0.001

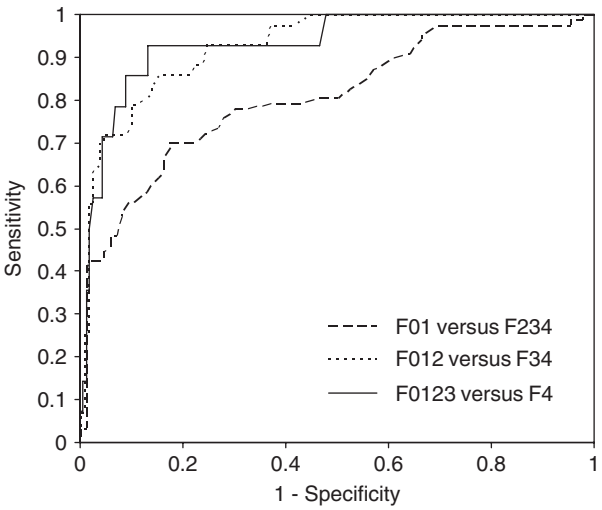

Fig. 2. Receiver-operating characteristic curves of liver stiffness for the diagnosis of significant fibrosis (F0, F1 vs F2, F3, F4), severe fibrosis (F0, F1, F2 vs F3, F4) and cirrhosis (F0, F1, F2, F3 vs F4) using liver biopsy as the reference.

moderate fibrosis to cirrhosis (F2, F3 or F4). The optimal cut-off values obtained in patients with chronic hepatitis C were 7.1, 9.5 and 12.5 kPa in Castera *et al*. (14) and 8.7, 9.6 and 14.5 kPa in Ziol *et al*. (16) for the diagnosis of $F \geq 2$, $F \geq 3$ and $F = 4$ respectively. In both cases, they were obtained by maximizing the sum of sensitivity and specificity. As there are numerous ways to chose cut-off values depending on the balance between sensitivity and specificity, we presented the cut-off values obtained by maximizing the sum of sensitivity and specificity and by maximizing the diagnosis accuracy (number of patients diagnosed properly). With both criteria, the cut-off value for the diagnosis of significant fibrosis ($F \geq 2$) was 7.2 kPa, which is similar to that obtained in HCV patients. However, for the diagnosis of severe fibrosis ($F \geq 3$) and cirrhosis ($F = 4$), cut-off values depended on the optimum criteria. Those obtained by maximizing the sum of sensitivity and specificity were slightly lower in this study than in HCV patients. This may suggest that fibrosis amounts are slightly lower in patients with chronic hepatitis B compared with patients with chronic hepatitis C, according to the METAVIR fibrosis scores. This could be because of the fact that HBV cirrhoses are more frequently of the macronodular type than HCV cirrhoses. These cut-off values are the first ones to be presented in a cohort of patients with chronic hepatitis B and need to be confirmed by additional studies.

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Table 5. Optimal cut-off values according to diagnosis question and optimum criteria

	F0, F1 vs F2, F3, F4	F0, F1, F2 vs F3, F4	F0, F1, F2, F3 vs F4
Maximum of sensitivity+specificity			
Cut off (kPa)	7.2	8.1	11.0
Sensitivity	70	86	93
Specificity	83	85	87
PPV	80	65	38
NPV	73	95	99
Likelihood ratio	4.0	5.6	7.0
Diagnosis accuracy	76	85	87
Maximum of diagnosis accuracy			
Cutoff (kPa)	7.2	10.5	18.2
Sensitivity	70	72	57
Specificity	83	95	97
PPV	80	84	67
NPV	73	91	96
Likelihood ratio	4.0	15.6	22.7
Diagnosis accuracy	76	90	94

NPV, negative predictive value; PPV, positive predictive value.

In this cohort of patients with chronic hepatitis B, LSM was reliable in the large majority of patients and was not recordable in 6.9%, which is comparable to the proportion of patients with non-interpretable LB (7.4%). The main reason for non-reliable results was, as in previous studies, related to overweight (24). The reproducibility of LSM, either intra- or interobserver, has been considered satisfactory previously (21, 25, 26).

Conversely, even if needle LB has been used as the 'gold standard' for the assessment of liver fibrosis, several authors have shown that a significant percentage of patients can be misclassified. When three percutaneous LBs were performed in the same patients using the same entry points, the overall concordance rate for cirrhosis, i.e. a histopathological feature of cirrhosis in all three biopsy specimens, was only 50% (27). Similarly, Abdi *et al.* (28) performed several post-mortem biopsies and showed that the diagnosis of cirrhosis could be obtained from one specimen in only 80% of cases. According to Bedossa *et al.* (29), sampling variation of liver fibrosis is an important limitation in the assessment of fibrosis with LB and it must be stressed that the median LB length in our study (16 mm), reflecting clinical practice, was far less than the optimal length defined by Bedossa *et al.* It is therefore possible that apparently false-positive results of LSM could be because of sampling error of the LB. However, as suggested by Rousselet *et al.* (30), the pathologist's level of experience has more influence on agreement than the length of the biopsy specimen. It seems reasonable to assess that LSM would provide a more accurate assessment of liver fibrosis than LB when interpreted by observers with a suboptimal experience in liver pathology. One may also hypothesize that misclassification in some cases may be related to the principle of LSM, which seems to accurately reflect liver fibrosis irrespective of its location and influence on liver architecture.

These results, combined with those of a study assessing the area of fibrosis measured by morphometry in patients who had LSM (M. Ziol, personal communication), suggest that elastography accurately reflects the amount of fibrosis, whether the cause of chronic hepatitis is either HBV or HCV. Obviously, the limitation of LSM is its inability to diagnose the grade of necroinflammatory activity or steatosis. However, steatosis is much less common in chronic hepatitis B than in chronic hepatitis C and contrary to chronic hepatitis C, the relevance of the assessment of steatosis in terms of prognosis and response to therapy in chronic hepatitis B has not been shown.

The association between liver stiffness and disease activity that we found in univariate analysis should be underlined as observed by Fraquelli *et al.* (25), who showed a step-wise increase of liver stiffness with necroinflammatory activity in a cohort of patients with disease of varied aetiology. Moreover, LSMs in patients with acute liver damage overestimate the real stage of fibrosis and may erroneously suggest the presence of liver cirrhosis (31, 32). The relationships between necroinflammatory activity and liver stiffness in patients with HBV infection and major changes of transaminases need further investigation (33). However, this association was not significant in the multivariate analysis, which would also suggest that the relationship between liver stiffness and activity in univariate analysis might be because of the fact that liver stiffness is correlated to fibrosis, which in turn is correlated to activity (Kendall $\tau_\beta = 0.41$, $P < 0.001$).

In conclusion, this study shows that LSM is an accurate method for the diagnosis of fibrosis stage in patients with chronic hepatitis B and may be a useful tool in the management of these patients in some conditions besides LB. These results must be validated externally in other large cohorts of patients.

Acknowledgements

We thank the teams of the five centres that participated in this study.

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