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ORIGINAL ARTICLE

Evaluation of Histological and non-Invasive Methods for the Detection of Liver Fibrosis: The Values of Histological and Digital Morphometric Analysis, Liver Stiffness Measurement and APRI Score

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Abstract Prognosis and treatment of liver diseases mainly depend on the precise evaluation of the fibrosis. Comparisons were made between the results of Metavir fibrosis scores and digital morphometric analyses (DMA), liver stiffness (LS) values and aminotransferase-platelet ratio (APRI) scores, respectively. Liver biopsy specimens stained with Sirius red and analysed by morphometry, LS and APRI measurements were taken from 96 patients with chronic liver diseases (56 cases of viral hepatitis, 22 cases of autoimmune- and 18 of mixed origin). The strongest correlation was observed between Metavir score and DMA (r=0.75 p<0.05), followed in decreasing order by LS and Metavir (r=0.61), LS and DMA (r=0.47) LS and APRI (r=0.35) and Metavir and APRI (r=0.24), respectively. DMA is a helpful additional tool for the histopathological evaluation of fibrosis, even when the sample size is small and especially in case of advanced fibrosis. The non-invasive methods showed good correlation with the histopathological methods; LS proved to be more accurate than APRI. The stronger correlation between LS values and Metavir scores, as well as the results of DMA in case of appropriate sample size were remarkable.

Keywords Liver fibrosis · Liver stiffness · Digital morphometric analysis · Liver biopsy

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Introduction

Liver fibrosis is considered a progressive process that could lead to complete remodelling of the liver and even to complications, some of which could be life-threatening and result in death. In the past two decades, the gold standard method for evaluation of fibrosis has been liver biopsy [1, 2], which is still the basis of diagnosis in the vast majority of chronic liver diseases today. Besides liver biopsy, histological assessment of the liver allows interpretation of the necroinflammation and judgement of the etiology [3].

Liver biopsy however has certain limitations. Being an invasive technique, it may rarely lead to serious complications (bleeding, pain, etc.) The biopsy site does not always represent the real status of the liver furthermore, the size of the sample, the number of portal tracts are not always sufficient for accurate diagnosis (sampling error) [1, 2]. The major limitation of the correct evaluation of liver samples is the decreasing number of qualified and experienced pathologists. On the other hand, if biopsy is taken by an experienced hepatologist, a number of valuable examinations can be carried out, such as computerized digital image analysis, immunohistochemical examinations as well as molecular tests.

Quantitative digital morphometric analysis (DMA) provides a standardized, objective evaluation of fibrosis offering digital images of liver biopsy samples, providing accurate

measurements of the collagen and remaining liver tissue areas, and calculating the proportion of the biopsy specimen that is occupied by collagen (collagen proportion area – CPA) [4, 5]. There are significant correlations between CPA and histological stage as well as CPA and liver stiffness (LS) or other serum markers of liver fibrosis and hepatic venosus pressure gradient [4–7].

In the present study, correlations were made between the DMA of liver fibrosis and Metavir scores as determined by individual pathologists, and LS values and APRI scores, respectively. Our study also assessed the influence of the liver's high histological inflammatory activity and fat content on the reliability of liver stiffness. In addition, the effect of sample size (number of portal tracts) on the correlation between histological analyses and non-invasive assessment of liver fibrosis was also analysed.

Material and Methods

Patients

Liver biopsy samples of 96 patients with chronic liver diseases from Hepatology Centers in Hungary (53 females, 43 males; age: 15–67 years, mean: 46.05 years) were examined retrospectively with the permission of the National Ethical Committee. The clinicopathological data of the patients are shown in Table 1.

Histological Evaluation

Biopsy samples were processed according to routine pathology procedures. In brief, the small, 1–3 cm long needle biopsy samples containing at least 5 portal tracts were submerged in 10 % neutral buffered formalin (in PBS, pH 7.0) and fixed for 24 h at room temperature. Following dehydration in a series of ethanol and xylene, samples were embedded in paraffin (FFPE samples). The 4–5 μm-thick sections were routinely stained with haematoxylin-eosin and Sirius red [8]. The stages of liver fibrosis (stages F0–F4) were evaluated using Metavir scores (F0=no fibrosis; F4=cirrhosis) [9, 10]. Histological evaluation was carried out by experienced hepatopathologists. The number of portal tracts in samples and the fat content in liver tissues (Grade 0: no fat, Grade 1: <33 %, Grade 2: 33–66 %, Grade 3: >66 %) [11] were also defined. The samples of hepatitis C virus (HCV) infected patients were scored based on the necroinflammation determined by the histological activity index (HAI) [12].

Liver Stiffness Evaluation (Transient Elastography, Fibroscan)

LS was measured (FibroScan 502, Echosens, Paris, France) in each case. The time between liver biopsy and LS evaluation was no more than 90 days, on average: 18.6 days.

Quantitative Digital Morphometric Analysis

Liver biopsies were stained with Sirius red. Quantitative analyses were performed on the tissue samples digitalized by Mirax Midi slide scanner equipped with a 20× Zeiss Plan-Apochromat objective and Marlin F146C camera (3DHistech Ltd, Budapest, Hungary). Areas of the whole sample and the designated fibrotic region were measured to calculate CPA. Measurements were made under preset, standardized circumstances using Leica QWin V3 morphometrical software (Leica Microsystem Imaging Solution Ltd., Cambridge, UK) (Fig. 1.a, b and c).

Biochemical Examinations

APRI score (based on ALT, AST, platelet count) was determined at the time of liver biopsy APRI=AST/ AST ULN/ platelet count (10^9/l)×100) [13].

Statistical Analysis

Correlations between the noted variables were determined using Spearman rank's correlation test. Differences were considered significant when $P<0.05$. All statistical analyses were performed using Statistica 9.0 (StatSoft Inc. Tulsa, OK) software program.

Results

Analysis of Samples

Significant positive correlation was verified between the results of the histological analyses and the non-invasive methods used to evaluate liver fibrosis in case of all 96 patients. The strongest correlation was found between Metavir scores and DMA (Fig. 2) ($r=0.75$, $p<0.05$) then, in descending order, between LS values and Metavir scores (Fig. 3) ($r=0.61$, $p<0.05$), LS values and DMA ($r=0.47$, $p<0.05$) (Fig. 4), LS values and APRI scores ($r=0.35$, $p<0.05$) and finally between Metavir and APRI scores ($r=0.24$ $p<0.05$), respectively.

Table 1 Clinicopathological data of patients

Etiology groups	No. of cases	Etiological subgroups	Fibrosis stage (METAVIR)	DMA %	LS (KPa)	APRI	ALT level (U/L)
Chronic viral	56	HCV (53)	F0 (4)	0.2–4.4	3.2–5.3	0.1–1.4	14–125
		HBV (3)	F1 (18)	0.6–10.7	3.8–11.9	0.1–2	10–152
			F2 (14)	2.3–10.6	4.2–20.4	0.1–13.2	12–376
			F3 (18)	0.8–25.9	5.6–23.9	0.1–22.6	12–319
			F4 (2)	18–25.1	20–24.3	1–3	27–62
Auto- immune	22	AIH (9)	F0 (4)	1.1–3.7	6.1–6.9	0.4–1.3	20–452
		PBC (6)	F1 (3)	0.8–5.6	5.3–7.6	0.1–0.1	10–20
		PSC (4)	F2 (5)	2.8–8.9	4.6–14.3	0.2–1.4	16–275
		overlap (3)	F3 (8)	5.1–37.3	6.1–18	0.1–1.8	17–196
			F4 (2)	12.6–33.4	20.6–45.7	0.2–1.9	226–83
Mixed origin	18	NAFLD (2)	F0 (4)	0.3–3.9	4.8–11.8	0.04–0.3	12–82
		ALD (5)	F1 (6)	1.5–9.2	3.7–37.4	0.25–1	18–271
		Crypt.(7)	F2 (3)	7.9–20	11.9–45	0.3–1.4	12–84
		Toxic (3)	F3 (2)	11–11.5	7.3–20.2	0.4–0.5	37–65
		Wilson (1)	F4 (2)	27.1–31	75	1–1.6	23–32
Total	96	–	F0 (12)	0.2–4.4	3.2–11.8	0.04–1.4	12–452
			F1 (27)	0.6–10.4	3.7–37.4	0.1–1	10–271
			F2 (23)	2.3–20	4.2–45	0.1–13.2	12–376
			F3 (28)	08–37.4	5.6–23.9	0.1–22.6	12–319
			F4 (6)	12.6–33.4	20–75	0,2–3	23–83

DMA digital morphometric analysis, *LS* liver stiffness, *APRI* aminotransferase to platelet ratio index, *ALT* alanine aminotransferase, *AIH* autoimmune hepatitis, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis, *HCV* hepatitis C virus, *HBV* hepatitis B virus, *ALD* alcoholic liver disease, *NAFLD* non-alcoholic fatty liver disease, *Crypt.* cryptogenic

Evaluation According to Fibrosis Stage

Significant positive correlation was observed between LS values and the results of DMA (r=0.47, p<0.05) and strong positive correlation (r=0.55, p<0.05) was also detected in the advanced stages of liver fibrosis (F3 and F4).

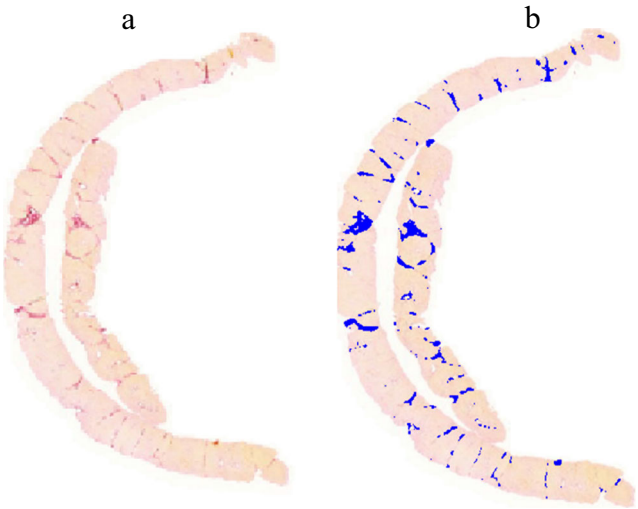

Fig. 1 Areas of both whole sample and designated fibrotic tissue were measured. The extent of fibrosis was expressed by the ratio of these. **a**: Liver biopsy sample stained with Sirius-red. **b**: Collagen proportional area is highlighted in blue

Evaluation According to Number of Portal Tracts

A substantial, though relatively weak correlation was found between LS values and results of DMA in cases in which less than 10 portal tracts were present (p<0.05, r=0.36), whereas strong correlation was demonstrable in cases which had large sample sizes (number of portal tracts was≥10), (p<0.05, r= 0.71). Significant positive correlation was notable between LS values and the results of DMA in cases showing advanced fibrosis (F3 and 4) with both low (<10) and high (≥10) numbers of portal tracts (r=0.55, p<0.05 and r=0.68, p<0.05,

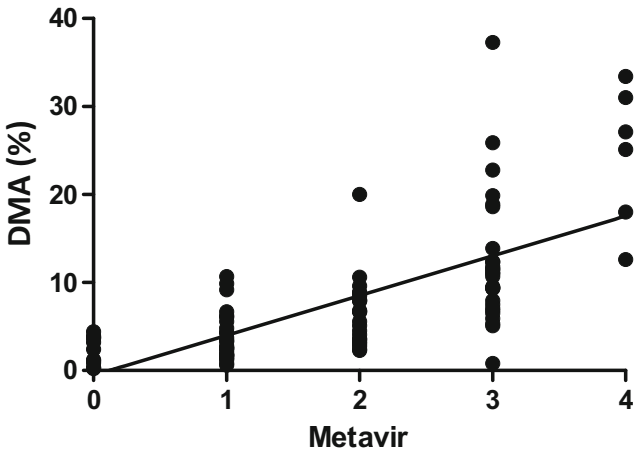

Fig. 2 Strong positive correlation was found between Metavir scores and digital morphometric analysis (DMA) (r=0.75, p<0.05)

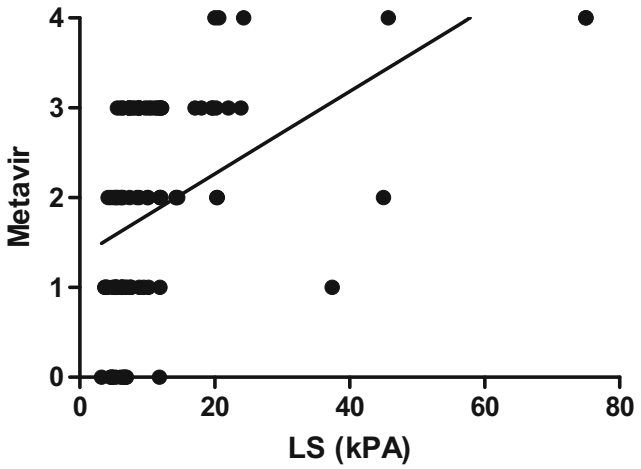

Fig. 3 Strong positive correlation was observed between Liver Stiffness (LS) values and Metavir scores (r=0.61, p<0.05)

respectively), but the correlation was stronger in cases which had large sample sizes.

Evaluation According to sex

The correlation between DMA and Metavir score was the same in both male and female patient groups (p<0.05, r=0.76), being similar between LS values and Metavir scores (males: p<0.05, r=0.66; females: p<0.05, r=0.58). The correlation between LS values and results of DMA was more pronounced in males as compared with females (males: r=0.65, females: r=0.34).

Role of fat Content of Liver Tissue

No differences were detectable between correlations of the DMA and Metavir score, LS values and Metavir score as well as LS values and results of DMA in cases of low-grade (Grades 0, 1) and high-grade steatosis (Grades 2–3), respectively (p<0.05, r=0.73; p<0.05, r=0.6; p<0.05, r=0.42 versus p<0.05, r=0.79; p<0.05, r=0.68; p<0.05, r=0.61, respectively).

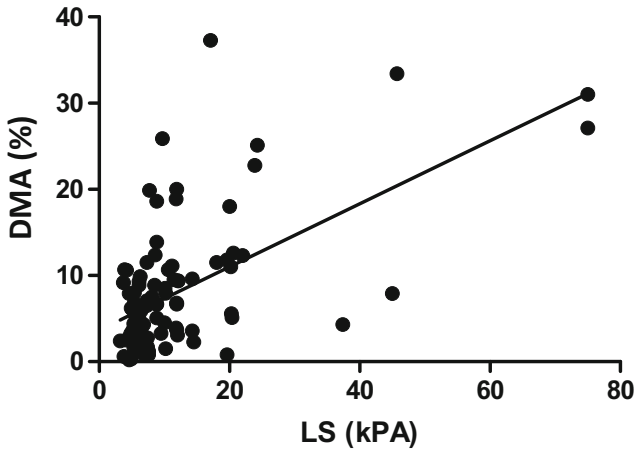

Fig. 4 Significant positive correlation was observed between Liver Stiffness (LS) values and results of the digital morphometric analysis (DMA) (r=0.47 p<0.05)

Evaluation of Results in Patients With Chronic Hepatitis C

The samples of 53 patients suffering from chronic hepatitis C were analysed separately, with the finding that correlation was the strongest between LS values and Metavir score (p<0.05, r=0.64). Similar correlation was found between LS values and results of DMA, as well as between LS values and APRI score (p<0.05, r=0.48).

Role of Histological Activity Index (HAI)

Out of 53 patients with chronic hepatitis C, 24 demonstrated necroinflammation (HAI levels >6). The correlations between the various methods used for evaluation of fibrosis were examined according to HAI level. No differences were found between DMA and Metavir score in the two groups (p<0.05, r=0.75). Furthermore, no relevant differences were observable between LS values and Metavir score in cases showing low (HAI ≤6) or high (HAI >6) inflammatory activity (p<0.05, r=0.50; p<0.05, r=0.64, respectively).

Discussion

Exact evaluation of liver fibrosis is essential for assessment of the natural history of the disease as well as for the interpretation of treatment effect [14]. New methods are available for the more accurate evaluation of the extent of liver fibrosis, but each method has its limitations. Currently, non-invasive techniques are being developed by reason of their lower risks and costs. Recently, the diagnosis of chronic viral hepatitis has rarely been made by means of liver biopsy. On the other hand, however, histological evaluation (liver biopsy) is necessary for the diagnosis of numerous liver diseases. Liver biopsy performed by an expert is safe, and histological evaluation of the liver tissue by an experienced pathologist could provide more information than any other non-invasive alternative. Another issue which could pose a problem is the matter of sampling errors; a sample may not be representative, the number of visible portal tracts may only be scarce [1, 2].

DMA has been used for several decades and has become a useful tool in the evaluation of the extent of liver fibrosis owing to the development of both hardwares (high-resolution segment scanner) and softwares [14–17]. Maduli et al. examined liver biopsy samples of patients with chronic hepatitis before and after antiviral treatment. The samples in their study were evaluated by two independent pathologists and the changes of liver fibrosis were assessed by DMA [18]. Their findings were similar to the observations made by

Arima et al., who evaluated liver samples of twenty-five patients suffering from chronic hepatitis C before and after interferon treatment. These authors were able to detect fibrosis regression in cirrhotic patients by means of DMA, which was otherwise undetectable by semi-quantitative method [19].

Many reports have been published in regard to the strong correlation between different semi-quantitative fibrosis scores and various quantitative morphometric methods used for the evaluation of fibrosis [17, 20–22]. In our study, we were able to detect good correlation between the results of DMA and Metavir score as well as LS values.

In our study, the samples containing low numbers of portal tracts were evaluated separately. Obviously, low sample size has negative influence on the results (sampling error). The weaker correlation that was observed between the results of DMA and LS values in this subgroup is supportive of the potential role of sampling errors. In line with the literary data, we also experienced DMA to be the most suitable for obtaining an accurate diagnosis in cases of advanced liver fibrosis and cirrhosis [21, 23, 24].

The differences between correlations of the examined parameters were also analysed according to sex. We found that correlation differed only between LS values and the results of DMA, being weaker among females. The most likely explanation for this result is that the liver tissue in females is less rigid, owing to the discrepant structure of their extracellular matrix (ECM) [25]. Furthermore, in rats it was found that ovarian hormones inhibit ECM formation by hepatic stellate cells [26].

Measurement of fibrosis by the methods used in our study was not influenced by either the presence or extent of liver steatosis. There are several publications on potential false positive LS results caused by moderate and severe steatosis. Interaction of liver fat and low-frequency vibration emitted by FibroScan is presumed, which may increase the noise and interfere with the LS measurement [27]. However, such influence was not observed by Arena et al. These authors examined liver fibrosis in 150 patients with chronic hepatitis C by transient elastography with the finding that accuracy of the measurement of LS was independent of the presence and extent of liver fat [28]. False positive LS values have been published in cases showing high inflammatory activity. However, the role of high biochemical activity (significantly elevated ALT levels) seems to be dominant as compared with HAI [29]. The reason for this alteration is not clear, but a possible explanation may be the observation that there is often no correlation between the biochemical activity (ALT level) and HAI [30]. In our study, the grade of HAI had no influence on the results of the morphometric evaluation of fibrosis and LS values.

Significant, but weak correlation was observed by us between APRI score and LS values (r=0.35) and Metavir score (r=0.24), respectively. The results were found to be similar in the HCV-infected subgroup (r=0.46 and r=0.27, respectively). Poynard et al. published a meta-analysis comparing different non-invasive fibrosis tests (Fibro-test, APRI, Fib-4, LS). The utility and reliability of these methods were tested in chronic hepatitis cases (HBV, HCV, ALD). The Fibro-test was the most reliable, but the APRI score also had significant prognostic value. According to these authors, the unreliability of tests containing transaminase levels (AST, ALT) is the consequence of the connection between necroinflammation activity and transaminase levels [31].

In line with the literary data, accuracy of the APRI score was the weakest in our study as well. Nevertheless, we consider it a useful method by reason of its simplicity, cheapness, unlimited applicability and its suitability for follow-up. False evaluation may be avoided by combination of this method with other non-invasive techniques and by taking the transaminase levels into account.

In conclusion, exact evaluation of fibrosis grade is of essential importance in determining prognosis, indicating and choosing the right method, as well as regarding both time and duration of treatment (especially in chronic hepatitis B and C) and efficacy of therapy. All these factors motivate the necessity of clarifying the histological techniques and the need to improve non-invasive methods. The latter are worthy of expansion since they are cost efficient and have low risk ratios. Precise assessment of the advantages and disadvantages, sensitivity and specificity of non-invasive methods is indispensable for their extensive application.

DMA is a helpful additional tool in the histopathological evaluation of fibrosis stage, and might also be of assistance to the less experienced pathologists in the field.

The tested non-invasive methods (APRI, LS) showed good correlation with the histopathological techniques.

These methods and their combination in particular, might well be useful for assisting the precise evaluation of fibrosis stage.

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