Diagnostic accuracy of computer morphometry for steatosis and fibrosis assessment in patients with chronic liver disease of various etiologies

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Abstract. Background. Accurate assessment of the fibrosis stage is crucial for effective treatment. Histological examination, the primary method used for assessing liver fibrosis, has certain limitations due to variation within each stage. Computer morphometry offers an objective and quantitative approach to complement histological analysis, providing additional diagnostic information. The purpose of this study was to analyze the computer morphometry data in patients with chronic liver diseases (CLD) of different etiologies and determine their diagnostic accuracy for liver fibrosis diagnosis.

Methods. Seventy-five patients with CLD, namely 24 with non-alcoholic fatty liver disease (NAFLD), 8 with alcoholic liver disease (ALD), 1 with toxic hepatitis, and 42 with chronic hepatitis C (CHC), were included in the study. Percutaneous liver biopsy was performed under ultrasound guidance using a semi-automatic needle Core Shot 16 G. The severity of fibrosis was assessed using the Metavir scale. For computer morphometry, biopsies were photographed and evaluated using the ImageJ 1.45S program (National Institutes of Health, USA). The computerized fibrosis index (CFI), steatosis index, and the number of apoptotic cells in 5 consecutive high-power fields were calculated. Receiver operating characteristic analysis was performed for CFI diagnostic accuracy assessment.

Results. Advanced liver fibrosis (F3-F4) was diagnosed in 62.5% of ALD cases and 31.0% of CHC patients. The highest CFI was found in ALD, exceeding the level of NAFLD and CHC patients by 3.3 (p < 0.01) and 2 times (p < 0.05), respectively. At the same time, people with NAFLD had the highest steatosis index (0.36 ± 0.11), which was 1.7 times higher (p < 0.05) than in ALD and CHC. Moreover, CFI correlated with the fibrosis stage (r = 0.71, p < 0.05). Stage I of liver fibrosis according to the Metavir scale is characterized by CFI up to 0.040, stage II — 0.041–0.130, stage III — 0.131–0.219, and stage IV — more than 0.220. CFI cut-off value was 0.017, which confirms the presence of liver fibrosis in patients with chronic liver diseases regardless of the etiology (sensitivity — 85.2%, specificity — 100.0%).

Conclusions. Computer morphometry significantly improves the accuracy and reliability of histological examination, and allows to objectify morphological assessment of liver steatosis and fibrosis to ensure long-term storage of the results.

Keywords: chronic liver diseases; computer morphometry; computerized fibrosis index; steatosis index

Introduction

Liver fibrosis and cirrhosis (K.74 in ICD-10) are severe liver diseases characterized by the partial irreversible replacement of the liver tissue with fibrous connective tissue or stroma [1–3]. In most cases, a fibrotic transformation of the liver is a consequence of chronic diseases such as viral hepatitis, autoimmune liver diseases, alcohol and non-alcoholic steatohepatitis, and biliary or metabolic disorders [4, 5].

It is important to establish the relationship between the stage of the liver disease and fibrosis before starting treatment, as this relationship directly affects the efficacy of therapy. Recent studies have shown that the amount of fibrous tissue in the liver correlates with hepatic venous pressure and can serve as a predictor of clinical decompensation [6].

Currently, histological examination remains the primary method for assessing and staging liver fibrosis [7, 8]. However, the challenges faced by pathologists lie in the certain variability within each stage. This is particularly evident in stage IV liver fibrosis, where the area of connective tissue and, consequently, the volume of functional tissue, can vary significantly. This, in turn, affects both the clinical manifestations of the disease and its prognosis.

For citation: Gastroenterologìa. 2023;57(2):85-89. doi: 10.22141/2308-2097.57.2.2023.536
Furthermore, a biopsy may lead to an inaccurate diagnosis if the disease affects only a part of the liver rather than the entire organ. However, most chronic liver diseases exhibit diffuse fibrotic changes. The reliability of a biopsy also depends on the sample size. Most studies on the reliability of liver biopsy have been conducted in patients with chronic hepatitis, as this disease warrants diagnostic biopsy. It has been recognized that a liver biopsy specimen measuring more than 1.5 cm in length with 6 to 8 portal tracts is sufficient for histological diagnosis. However, the width of the biopsy specimen and the type of needle used also play a significant role due to the unique architecture of the liver [2]. According to most researchers, subcapsular biopsy material contains more fibrous tissue than profound layers [7]. Moreover, morphological diagnosis is impossible or significantly complicated in cases of macronodular or mixed cirrhosis, as large nodules (which can reach a diameter of 5 cm) do not contain fibrous septa inside.

Due to the fact that fibrosis development plays an important role in the course of liver diseases, the quantitative assessment of the fibrosis stage becomes a priority for scientists and practical morphologists. It contributes to the understanding of the clinical significance of extracellular matrix excessive deposition.

The main method for liver fibrosis quantitative assessment is morphometry, which primarily involves the examination of biopsy material obtained through percutaneous biopsy [10–12]. This technique allows for unbiased evaluation of changes and enables statistical comparison with the results of other diagnostic methods (biochemical, biophysical, physiological, etc.) [13]. Additionally, storing research results for morphological examination. Biopsy samples were photographed and measurements were performed using the ImageJ 1.45S software (developed at the National Institutes of Health, USA). The CFI (the ratio of the fibrotic tissue area to the total biopsy specimen area), steatosis index (the number of hepatocytes with fatty degeneration per 100 cells), and the number of apoptotic cells in 5 consecutive high-power fields were calculated.

Statistical analysis of the obtained data was conducted using the Statistica 10.0 software. The mean (M), standard error of the mean (Me), lower and upper quartiles (Q1 and Q3) were calculated. The comparison of median values of variables was performed using the Mann-Whitney U test and Kruskal-Wallis test. Differences were considered significant at p < 0.05. To assess the diagnostic accuracy of the parameter, the analysis of ROC curves was applied. The threshold value, sensitivity, specificity, area under the ROC curve (AUC), and its 95% confidence interval (CI) were calculated.

Results and discussion

The distribution of all cases by fibrosis stage (according to Metavir) is presented in Fig. 1, which shows that advanced liver fibrosis (F3–F4) was diagnosed in 62.5% of ALD cases and 31.0% of CHC cases. Liver fibrosis was absent in 14 (18.7%) patients with chronic liver diseases.

According to morphometry data, apoptotic cells were observed in half of the patients. The highest number of apoptotic cells, ranging from 5 per high-power field (HPF), was found in chronic hepatitis C patients, followed by 5–12 per HPF in NAFLD patients, and more than 12 per HPF in ALD patients (Fig. 2).

The highest steatosis index was found in the NAFLD group (0.36 ± 0.11), which corresponds to the morphological findings regarding the extent of liver tissue involvement in

<table>
<thead>
<tr>
<th>Fibrosis stage</th>
<th>Morphological features</th>
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<tbody>
<tr>
<td>Normal structure</td>
<td>No fibrosis</td>
</tr>
<tr>
<td>Stage I</td>
<td>Portal fibrosis without septa</td>
</tr>
<tr>
<td>Stage II</td>
<td>Fibrosis with rare septa</td>
</tr>
<tr>
<td>Stage III</td>
<td>Numerous septa without cirrhosis</td>
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<tr>
<td>Stage IV</td>
<td>Cirrhosis</td>
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</table>

Computer morphometry was used as an additional method for the morphological examination. Biopsy samples were photographed and measurements were performed using the ImageJ 1.45S software (developed at the National Institutes of Health, USA). The CFI (the ratio of the fibrotic tissue area to the total biopsy specimen area), steatosis index (the number of hepatocytes with fatty degeneration per 100 cells), and the number of apoptotic cells in 5 consecutive high-power fields were calculated.
fatty liver disease. In contrast, the fibrosis index in this group of patients was the lowest, with a value of (0.04 ± 0.01) (Table 2).

In the CHC group, the most significant variations in the steatosis index were observed, with a mean value of (0.23 ± 0.03), while the fibrosis index was (0.08 ± 0.02). The ALD group significantly differed from the NAFLD group in terms of the fibrosis index (0.13 ± 0.01, p < 0.01) and steatosis index (0.21 ± 0.04, p < 0.05).

A significant strong correlation was found between the subjective assessment of liver fibrosis stage according to Metavir and the absolute value of the fibrosis area index (r = 0.71; p < 0.05) in patients with CLD.

At the first stage of fibrosis, the connective tissue appeared as small periportal nodules (Fig. 3).

In 15.4 % of cases, the portal tracts were slightly expanded, and the CFI was within the range of up to 0.040 (Table 3).

The second stage of fibrosis was characterized by the beginning of incomplete portal-portal septa formation, and the periportal area consisted of dense connective tissue infiltrated by lymphocytes and plasma cells. The CFI ranged from 0.041 to 0.130. The third stage of fibrosis was characterized by the expansion of perisinusoidal fibrosis, accumulation of connective tissue around portal tracts and central vein, and the presence of complete and incomplete portal-portal and

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**Table 2 — Morphometric parameters in patients with chronic liver diseases**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NAFLD (n = 24)</th>
<th>CHC (n = 42)</th>
<th>ALD (n = 8)</th>
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</thead>
<tbody>
<tr>
<td>CFI</td>
<td>0.04 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.13 ± 0.01***</td>
</tr>
<tr>
<td>Steatosis index</td>
<td>0.36 ± 0.05</td>
<td>0.23 ± 0.03*</td>
<td>0.21 ± 0.04*</td>
</tr>
</tbody>
</table>

Notes: * — p < 0.05; ** — p < 0.01 — significance of differences compared to NAFLD; † — p < 0.05 — significance of differences compared to CHC.
portal-central septa. The CFI ranged from 0.131 to 0.219. In the fourth stage of fibrosis, the lobular structure of the liver was significantly disrupted, complete portal-portal and portal-central septa were observed, along with the formation of micro- and macronodular structures (nodules). The CFI was above 0.220.

The overall CFI value in ALD patients was in 5.2 times (p < 0.05) higher than in the NAFLD group and 2.5 times higher than in the CHC group.

In patients with liver fibrosis, the median CFI was 0.0523 (0.0230; 0.1774), while in patients without liver fibrosis, this value was 18 times lower, at 0.0028 (0.0023; 0.0036) — according to the Kruskal-Wallis test, the likelihood of the difference was lower than 0.0001 (Fig. 4).

ROC analysis demonstrated a high area under the ROC curve (AUC) for the CFI which was 0.977 (95% CI 0.912–0.998) (p < 0.0001). Also, CFI can be used to assess the risk of liver fibrosis in patients with chronic liver diseases. The threshold value of CFI, which classifies patients with CLD into the fibrosis group, was 0.017 (sensitivity — 85.2 %, specificity — 100.0 %) (Fig. 5).

Therefore, the quantitative assessment of steatosis and fibrosis on liver biopsies by computer morphometry is a highly specific method, promising for evaluating the efficacy of treatment and confirming the reliability of subjective methods of morphological staging.

Conclusions

1. The computer morphometry of liver biopsies significantly improves the accuracy and reliability of histological examination, objectifies the process of morphological assessment of steatosis and fibrosis, and ensures long-term storage of results data.

2. The CFI is higher in ALD patients compared to NAFLD patients (3.3 times higher; p < 0.01) and CHC patients (2 times higher; p < 0.05). Meanwhile, the steatosis index was highest in the NAFLD group (0.36 ± 0.11), which was 1.7 times higher (p < 0.05) compared to the values in the ALD and CHC groups.

3. The CFI correlates with the stage of fibrosis (r = 0.71, p < 0.05). For the first stage of liver fibrosis according to the Metavir scale, CFI values are typically within the range of up to 0.040. For the second stage, the range is 0.041–0.130. For the third stage, it is 0.131–0.219, and for the fourth stage, it is 0.220 and above.

4. A CFI above 0.017 confirms the presence of liver fibrosis in patients with chronic liver diseases regardless of the etiology of the disease (sensitivity — 85.2 %, specificity — 100.0 %).

References


**Діагностичне значення показників комп’ютерної морфометрії щодо оцінки стеатозу та фіброзу в інших хронічних дифузних захворюваннях печінки (ЖДЗП)**

**Резюме. Актуальність.** Точна оцінка стадії фіброзу має вирішальне значення для ефективного лікування. Гістологічне дослідження — основний метод, що використовується для оцінки фіброзу печінки, — має певні обмеження через варіацію. Комп’ютерна морфометрія пропонує об’єктивізувати процес морфологічної оцінки стеатозу та фіброзу печінки. 

**Мета.** Розглянути аспект діагностики стеатозу та фіброзу печінки при хронічних дифузних захворюваннях печінки за допомогою комп’ютерної морфометрії.

**Методи.** Для перевірки гіпотези зроблено вивчення фіброзу печінки в ознаках різних етіологічних факторів. Комп’ютерна морфометрія була здійснена за допомогою програми ImageJ 1.45S (робота виконана в National Institutes of Health, USA). Розраховані колірні індекси фіброзу (КіФ) — показники, що відображають індивідуальну середню кількість змінених клітин, а також індекс стеатозу.

**Результати.** Вивчення виявило значну різницю в оцінці фіброзу печінки, залежно від етіologies факторів. КіФ була вищим в групі ХГС порівняно з АХП у 3,3 раза (р < 0,05). Водночас індекс стеатозу був вищим в групі ХГС порівняно з АХП у 6,2 раза (р = 0,01). При цьому показники були досить відхилені від контрольних значень.

**Висновки.** Комп’ютерна морфометрія виявляється як досить корисний метод для оцінки фіброзу печінки, інтенсивності стеатозу та фіброзу в інших хронічних дифузних захворюваннях печінки.

**Ключові слова:** комп’ютерна морфометрія, оцінка стеатозу та фіброзу печінки, етіологічні фактори.

**Автори.** О.І. Диденко, О.П. Петишко

**Інформація про авторів.** О.І. Диденко — концепт-редагатор; О.П. Петишко — дизайнер та редактор.

**Інформація про фінансове забезпечення.** Робота виконана в рамках дослідницького проекту "Медіко-алгоритмічна система".

**Прийнято до друку 19.04.2023.**