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Fibroscan and non-invasive indices for the diagnosis of nonalcoholic fatty liver disease

Abstract. Background. Non-alcoholic fatty liver disease (NAFLD), an independent nosological entity, is characterized by fat accumulation in hepatocytes not associated with alcohol abuse, and includes a wide spectrum of disorders: from fatty liver, non-alcoholic steatohepatitis to fibrosis with possible outcome in liver cirrhosis. Given the prevalence of this disease, the deterioration of the quality of life of patients, increased mortality from complications, there is a growing interest in developing techniques for accurate and timely assessment of fibrosis. **Objective:** comparative characteristics of the results of transient elastometry (FibroScan) and non-invasive laboratory indices in the determination of fibrotic transformation of the liver in patients with non-alcoholic fatty liver disease. **Materials and methods.** The study included patients with NAFLD, who underwent diagnostics and treatment in the department of liver and pancreas of the SI "Institute of Gastroenterology of the NAMS of Ukraine". **Results.** We have examined 42 patients with NAFLD, among which 18 (45 %) men and 24 (55 %) women. All patients underwent calculation of non-invasive markers of liver fibrosis: aspartate aminotransferase to platelet ratio index (APRI), fibrosis-4 index, aspartate aminotransferase/alanine aminotransferase ratio, the measurement of liver stiffness using the FibroScan apparatus. **Conclusions.** Our results are consistent with most studies indicating that the most effective non-invasive index is APRI. The combination of transient elastography (FibroScan) and the APRI index may provide a more effective approach to the diagnosis of liver fibrosis in patients with NAFLD.

Keywords: non-alcoholic fatty liver disease; liver fibrosis; non-invasive diagnostic methods; transient elastography

Introduction

Nonalcoholic fatty liver disease is a condition of excess fat in the hepatic parenchyma in the absence of significant alcohol consumption. The boundary value is 5 % fatty inclusions according to the morphological study, or 5.6 % according to the results of magnetic resonance spectroscopy [1]. NFLD is a worldwide problem with prevalence according to various studies from 12.5 % to 51 %. The scope of these indicators is due to the presence of various risk factors and depends on the methods of diagnosis [1–4]. The spectrum of pathology included in the concept of NFLD consists of simple steatosis, steatohepatitis with the possibility of progression to cirrhosis of the liver and even hepatocellular carcinoma. Recently, there is an understanding that NFLD is a hepatic embodiment of the metabolic syndrome and is closely related to insulin resistance, the risk of car-

diovascular pathology and the development of diabetes mellitus. Rapid progression of fibrosis is a significant problem, although it occurs in a small number of patients with fatty disease. A gold standard for isolating a group of patients at risk of disease progression and to determine the degree of fibrosis is still considered a morphological study, although liver biopsy is associated with certain inconveniences and life-threatening complications [5].

In the last decade, alternative methods of noninvasive or minimally invasive determination of the degree of fibrosis with various liver pathologies, including NFLD, are actively developing. Among them, the evaluation of various indices calculated on the basis of blood values — the ratio of activity of aspartate aminotransferase (AST) to the number of platelets (APRI), the ratio of aspartate aminotransferase to alanine aminotransferase (AST/ALT), commercial integral

indices — NAFLD fibrosis score (NFS), Fibrosis 4 calculator (FIB-4) [1]. In the diagnosis of NFLD, imaging methods are becoming increasingly important. Ultrasound, computer and magnetic resonance imaging, magnetic resonance spectroscopy, and transient elastography are used. Computed tomography is used in a limited way because of both the radiation load and lack of sensitivity.

Transient elastography (TE) measures the propagation velocity of transverse waves at a depth of 25–65 mm and is converted to a liver stiffness index (LSM). The resistance of deformation, which depends on the rigidity of the liver, is expressed by the Young's modulus in kilopascals (kPa). The TE uses the formula $E = 3pV^2$, which is based on Hooke's law, where E is Young's modulus, p is the density (presumably 1000 kg/m³) and V is the velocity of propagation of transverse waves [6].

The advantages of TE are simple, non-invasive, as well as high clinical significance. Restrictions on the use of the method are the presence of ascites in the patient, excessively developed fatty tissue, narrow intercostal spaces. The effectiveness of TE in patients with viral hepatitis has already been confirmed by a large number of studies [7, 8]. At the same time, the diagnostic capabilities of the method in patients with NFLD have not been adequately described.

The purpose. Comparative characteristics of the results of transient elastometry (FibroScan) and noninvasive laboratory indices in the determination of fibrous liver transformation in patients with NFLD.

Materials and methods

The study included patients with NFLD who underwent examination and treatment in the Department of Liver and Pancreatic Diseases at the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine. The diagnosis was based on ultrasound examination of the abdominal and evaluation of the activity of liver enzymes. Patients with a different liver disease, including viral, drug, autoimmune and alcoholic hepatitis, were excluded from the study.

The activity of ALT and AST in blood serum was determined by the colorimetric dinitrophenylhydrazed Reitman-Frenkel method. The laboratory reference range of ALT and AST was up to 40 U/L, and the normal platelet count was 150–450 g/l.

The liver stiffness measurement (LSM) was performed on a FibroScan 502 Touch F 60156 machine, the company Echosens (France). The impact of the sensor pin was applied to the right intercostal space at the level of the anterior or middle axillaries line and was directed to the right lobe of the liver. Using ultrasonic M- and A-mode, the shear wave propagation velocity and the controlled ultrasonic attenuation parameter were estimated through a standard 4 cm section. The final result was expressed in kPa and was the median value of 10 individual actual measurements. The studies, which resulted in 10 valid measurements with a valid level of at least 60 % and an interquartile range of no more than 30 % of the median stiffness values, were considered successful. The results were evaluated as follows: F 0 — 0–5.9 kPa, F 1 — 6–6.9 kPa, F 2 — 7–9 kPa, F 3 — 9.1–10.3 kPa, F 4 — 10.4 kPa and more.

The AST/ALT ratio was calculated for each patient.

APRI was calculated by dividing the AST level [U/L], expressed as the number of times above the upper limit of normal [ULN], by platelet count [G/L]:

$$APRI = (AST [U/L] \times 100) / (AST [ULN] \times \text{platelet count [G/L]}).$$

FIB-4 was calculated using the formula:

$$FIB-4 = (age [years] \times AST [U/L]) / (\text{platelet count [G/L]} \times \sqrt{ALT [U/L]}).$$

The statistical analysis was carried out with the Statistica for Windows 6.0. Since most of the data had a normal distribution, parametric statistics were used — mean (M) and standard deviation (SD). Correlation analysis was used to reveal the interrelations between different values of the investigated indicators. To determine the significance of the differences between the integral indices of fibrosis of AST/ALT, APRI and FIB-4 in patients with minimal (F 0–1), moderate (F 2–3) and severe (F 4) fibrosis, the Student's t-test was applied. The difference was considered significant when $p < 0.05$.

Results

42 patients with NFLD were examined, including 18 (45 %) men and 24 (55 %) women; the average age of patients was 44.9 ± 1.6 years.

There were no significant differences in age, platelet count, ALT and AST activity between men and women. The results of a biochemical blood test and platelet count are shown in Table 1.

The average liver stiffness index was (9.10 ± 1.33) kPa and in most patients there was no fibrosis (F0 — 31 %) or fibrosis was moderately expressed (F1/2 — 45.3 %). In male patients, the stiffness index was higher than in women, but this difference was not significant (11.7 (SD: 12.7) compared with 7.1 (SD: 2.22), respectively, $p = 0.091$). According to TE, the patients were divided into 3 groups: 1 — with minimal fibrosis (F 0–1), 2 — with moderate fibrosis (F 2–3) and 3 — with severe liver fibrosis.

Correlation analysis revealed a positive relationship between ALT level and liver stiffness ($r = 0.32$ and 0.47 for $p = 0.003$ and 0.002 for Kendall and Spearman correlations, respectively), as well as AST level and liver stiffness ($r = 0.39$ and 0.55 at $p = 0.0002$ and 0.0001 for Kendall and Spearman correlations, respectively). There was also a positive correlation between APRI and transient elastometry ($r = 0.33$ and 0.49 for $p = 0.002$ and 0.001 for Kendall and Spearman correlations, respectively). There was a significant difference in the APRI score between patients with moderate

Table 1 — Platelet and hepatic enzyme indices in the examined patients

Lab. test	M ± SD	Normal range
Platelets, g/l	252.47 ± 66.82	150–450
Serum ALT, U/L	68.54 ± 56.81	≤ 40
Serum AST, U/L	46.98 ± 32.74	≤ 40

to severe (F 2–3/F 4), and initial and severe (F 0–1/F 4) fibrosis (Table 3).

A weak correlation between FC, APRI, FIB-4 and AST/ALT ratio can be explained by the small number of patients examined, which necessitates continuation of this study, as well as further monitoring of patients at the stages of NFLD development.

Discussion

The pathophysiology of a specific disease lies at the heart of the development of biomarkers, reflecting the different stages of the development of this disease. In the case of NFLD, there are two potential targets for researchers. The first is the introduction of markers in practice, by which one could distinguish simple steatosis from steatohepatitis — a state with a more serious prognosis. The second goal is to identify the stage of fibrosis. Most prospective cohort studies of patients with NFLD showed that the prognosis is determined by the stage and level of progression of fibrosis even more than by the presence of necrotic inflammation. Clinical significance is the possibility of differentiation between absence or minimal fibrosis (F 0–1), significant fibrosis (F 2), severe fibrosis (F 3) and cirrhosis (F 4) [9].

F.C. Kruger, C.R. Daniels, M. Kidd and colleagues evaluated the results of 111 patients with histologically proven fatty liver disease. Biopsy specimens were described according to the NASH clinical research network (CRN) criteria. Groups with steatosis, steatohepatitis with absent or moderate fibrosis and with severe fibrosis were identified. The sensitivity and specificity of APRI with NFS and ALT/AST ratio were compared. The APRI value was significantly

Table 2 — Distribution of different degrees of fibrosis in the examined patients according to transient elastometry

The stage of fibrosis (kPa)	N (%)
F 0 (≤ 5.9)	13 (31)
F 1 (6–6.9)	7 (16.7)
F 2 (7.0–9.0)	12 (28.6)
F 3 (9.1–10.3)	3 (7)
F 4 (≥ 10.4)	7 (16.7)
All patients	42 (100)

Table 3 — Integral indicators of liver fibrosis and the reliability of differences between them in patients, distributed depending on the TE

Tests	Group 1 (N = 20)	Group 2 (N = 15)	Group 3 (N = 7)
LSM, kPa	5.13 ± 1.36*	8.30 ± 0.82*	22.14 ± 15.96
FIB-4	0.98 ± 0.69	1.08 ± 0.33	1.60 ± 1.10
APRI	0.37 ± 0.24	0.44 ± 0.21*	0.998 ± 0.550
AST/ALT	0.78 ± 0.28	0.71 ± 0.20	0.96 ± 0.45

Note. * — $p < 0.001$.

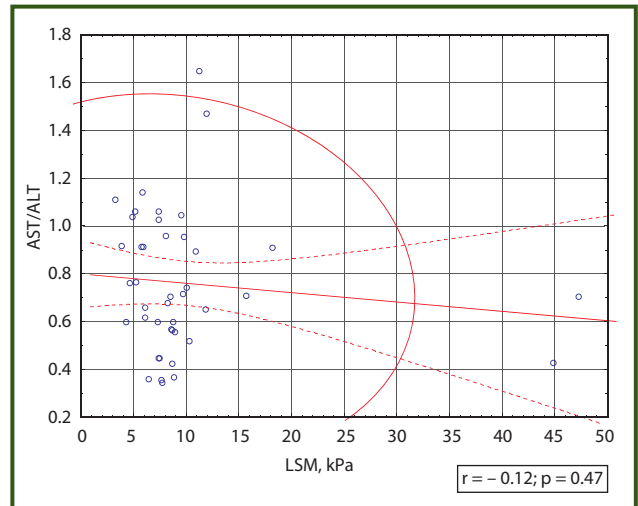


Figure 1 — Correlation between the FibroScan (liver stiffness, kPa) and the AST/ALT ratio

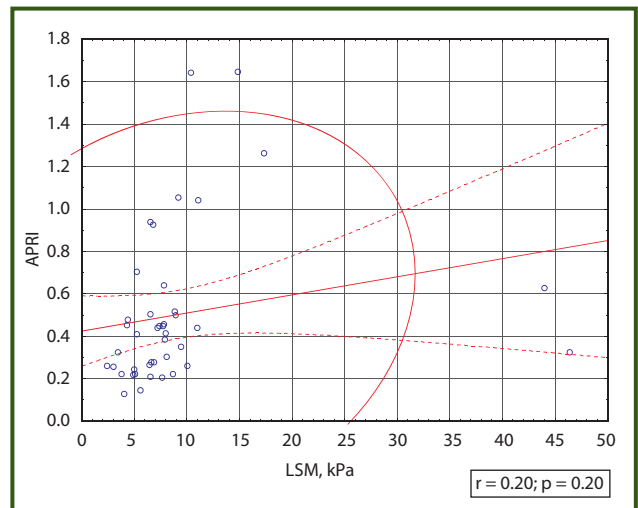


Figure 2 — Correlation between the FibroScan (liver stiffness, kPa) and the APRI

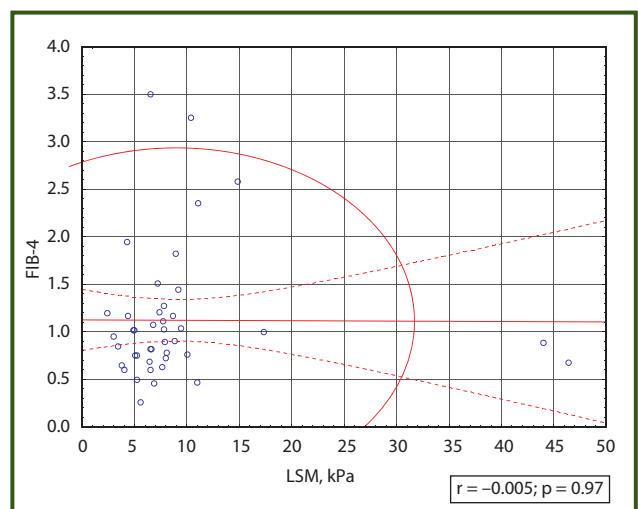


Figure 3 — Correlation between the FibroScan (liver stiffness, kPa) and the FIB-4

higher with severe fibrosis. So the optimal cut-off point was 0.98 with a sensitivity of 75 % and a specificity of 86 %. The NFS for steatohepatitis was significantly lower in the group with severe fibrosis. Positive predictive value was 54 % for APRI, while for NFS it was 34 %. The negative predictive value was 93 % for APRI and 94 % for NFS. Analysis of the data showed that for the diagnosis of severe fibrosis APRI is more preferable than NFS and ALT/AST [10].

A group of American scientists retrospectively analyzed a database of 514 adult patients with NFLD, assessing the diagnostic accuracy of FIB-4, comparing it with seven other non-invasive markers. The authors concluded that FIB-4 is superior to other fibrosis indices in patients with NFLD, but there is still a need to develop more sensitive non-invasive markers [11].

English scientists S. McPherson, S.F. Stewart, E. Henderson et al. compared morphology data of 145 Newcastle Hospitals Fatty Liver Clinic patients over a 6-year period. The FIB-4 scale had the best diagnostic accuracy for severe fibrosis — AUROC = 0.86, the AST/ALT ratio (AUROC = 0.83), NFS (AUROC = 0.81), and the AST/platelet ratio (AUROC = 0.67). AST/ALT, FIB-4 and NFS had a negative predictive value above 90 %. The positive prognostic value was moderate. To exclude severe hepatic fibrosis, biopsies can potentially be avoided in 69 % of patients with AST/ALT, 62 % with FIB-4, 52 % with NFS [12].

The French P. Calès, F. Lainé, J. Boursier compared the NFLD-specific certified FibroMeter and NFS tests with the non-specific APRI test. The data of 235 patients with fatty liver disease of two clinical centers were evaluated. The highest accuracy was 91 % with a marked fibrosis in FibroMeter, whose AUROC was 0.94, which was significantly higher than in NFS (0.884, $p = 0.008$) and APRI (0.866, $p < 0.001$). Using threshold values of 90% predictive value, liver biopsy could be avoided in most patients: FibroMeter — 97.4 %, NFS: 86.8 % ($p < 0.001$) and APRI: 80.0% ($p < 0.001$) [13].

Conclusions

In our study, the standard with which we compared the minimally invasive markers of fibrosis was transient elastometry. According to the results of many years of clinical practice, TE measurement by FibroScan is a safe method allowing to determine the degree of fibrosis with high accuracy. The results of our work are consistent with most studies, according to which the most effective of minimally invasive indices is APRI. With its help, it is possible to differentiate the stage of fibrosis with high accuracy (from moderate — F 2–3 to severe F 4), but its use is limited in the diagnosis of initial fibrosis (F 1). The combination of transient elastometry (FibroScan) and the APRI index can provide a more efficient approach in the diagnosis of liver fibrosis in patients with NFLD. Thus, the use of TE with FibroScan in combination with the APRI index allows early diagnosis of fibrosis as an alternative to puncture liver biopsy.

Conflicts of interests. Authors declare the absence of any conflicts of interests that might be construed to influence the results or interpretation of their manuscript.

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FibroScan і неінвазивні індекси в діагностиці неалкогольної жирової хвороби печінки

Резюме. *Актуальність.* Неалкогольна жирова хвороба печінки (НЖХП) є самостійною нозологічною одиницею, характеризується накопиченням жиру в гепатоцитах, не пов'язаним зі зловживанням алкоголем, і включає широкий спектр порушень — від жирової дистрофії печінки, неалкогольного стеатогепатиту до фіброзу з можливим переходом у цироз печінки. З огляду на поширеність цієї патології, погіршення якості життя хворих, збільшення смертності від ускладнень зростає інтерес до розробки методів для точної й своєчасної оцінки фіброзу. **Мета:** порівняльна характеристика результатів транз'єнтної еластометрії (FibroScan) і неінвазивних лабораторних індексів у визначенні фіброзної трансформації печінки у хворих із неалкогольною жировою хворобою печінки. **Матеріали та методи.** У дослідження включені пацієнти з НЖХП, які проходили обстеження й лікування у відділенні захворювань

печінки та підшлункової залози ДУ «Інститут гастроентерології НАМН України». Обстежені 42 пацієнти з НЖХП, серед яких 18 (45 %) чоловіків і 24 (55 %) жінки. Усім пацієнтам було виконано розрахунок неінвазивних маркерів фіброзу печінки: APRI, FIB-4, співвідношення аланінамінотрансферази/аспартатамінотрансферази, проведено вимірювання жорсткості печінки за допомогою апарату FibroScan. **Результати** нашої роботи узгоджуються з більшістю досліджень, згідно з якими найбільш ефективним з малоінвазивних індексів є APRI. **Висновки.** Поєднання транз'єнтної еластометрії (FibroScan) з індексом APRI може забезпечити більш ефективний підхід до діагностики фіброзу печінки у хворих з НЖХП.

Ключові слова: неалкогольна жирова хвороба печінки; фіброз печінки; неінвазивні методи діагностики; транз'єнтна еластометрія

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FibroScan и неинвазивные индексы в диагностике неалкогольной жировой болезни печени

Резюме. *Актуальность.* Неалкогольная жировая болезнь печени (НЖБП) является самостоятельной нозологической единицей, характеризуется накоплением жира в гепатоцитах, не связанным со злоупотреблением алкоголем, и включает широкий спектр нарушений — от жировой дистрофии печени, неалкогольного стеатогепатита до фиброза с возможным исходом в цирроз печени. С учетом распространенности этой патологии, ухудшения качества жизни больных, увеличения смертности от осложнений растет интерес к разработке методов для точной и своевременной оценки фиброза. **Цель:** сравнительная характеристика результатов транз'єнтной эластометрии (FibroScan) и неинвазивных лабораторных индексов в определении фиброзной трансформации печени у больных с НЖБП. **Материалы и методы.** В исследование включены пациенты с НЖБП, которые проходили обследование и лечение в отделении заболеваний печени

и поджелудочной железы ГУ «Институт гастроэнтерологии НАМН Украины». Обследовано 42 пациента с НЖБП, среди которых 18 (45 %) мужчин и 24 (55 %) женщины. Всем пациентам был выполнен расчет неинвазивных маркеров фиброза печени: APRI, FIB-4, соотношение аланинаминотрансферазы/аспартатаминотрансферазы, проведено измерение жесткости печени при помощи аппарата FibroScan. **Результаты** нашей работы согласуются с большинством исследований, согласно которым наиболее эффективным из малоинвазивных индексов является APRI. **Выводы.** Сочетание транз'єнтной эластометрии (FibroScan) с индексом APRI может обеспечить более эффективный подход в диагностике фиброза печени у больных с НЖБП.

Ключевые слова: неалкогольная жировая болезнь печени; фиброз печени; неинвазивные методы диагностики; транз'єнтная эластография