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Transient elastography for noninvasive assessment of liver fibrosis in patients with primary biliary cirrhosis

Tranzijentna elastografija u neinvazivnoj proceni fibroze jetre kod bolesnika sa primarnom bilijarnom cirozom

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Abstract

Backgrund/Aim. In recent decades noninvasive methods for the assessment and monitoring of liver fibrosis have been developed and evaluated in numerous chronic liver diseases. The aim of this study was to evaluate the diagnostic accuracy of noninvasive markers for fibrosis assessment transient elastography (TE) and biochemical markers using liver biopsy as reference in patients with primary biliary cirrhosis (PBC). **Methods.** One hundred and twenty-two patients underwent both liver biopsy and blood tests on the same day and TE in a month following the biopsy and the tests. Liver biopsies were reviewed by a single pathologist using the METAVIR scoring system for assessment of liver fibrosis. Aspartate aminotransferase (AST), platelet ratio index (APRI), Forns scores, AST and alanine transaminase (ALT) ratio and TE were compared with liver fibrosis stage in order to determine the best noninvasive marker of liver fibrosis. **Results.** There was a statistically significant difference ($p < 0.05$) for the APRI score, Forns index and TE according to stages of liver fibrosis. TE showed superior diagnostic performance when compared to other surrogate markers of liver fibrosis that were investigated. Optimal cut-off for TE were 4.25 and 5.9 kPa for diagnosing the presence of fibrosis and distinguishing mild/moderate and advanced stages of fibrosis respectively. The areas under the receiver operating characteristic (AUROC) of TE were 0.963 and 0.865, respectively. **Conclusion.** Based on our investigation the APRI score, Forns index and TE adequately predict fibrosis stage in patients with primary biliary cirrhosis, but the most sensitive and specific parameter appears to be TE. Using noninvasive markers and methods in the evaluation of patients in daily clinical practice may reduce, but not eliminate, the need for invasive diagnostic procedures.

Key words:
liver cirrhosis; biopsy; blood chemical analysis; biological markers; elasticity imagine techniques; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Prethodnih decenija otkrivene su neinvanzivne metode za procenu i praćenje fibroze jetre kod hroničnih bolesti jetre. Cilj ove studije bila je procena dijagnostičke preciznosti neinvanzivnih metoda za određivanje fibroze jetre [tranzijentna elastografija (TE) i biohemijski markeri], pri čemu je kao zlatni standard korišćena biopsija jetre kod bolesnika sa primarnom bilijarnom cirozom. **Metode.** U studiju su bila uključena 122 bolesnika kod kojih su istog dana urađene biohemijske analize i biopsija jetre, a mesec dana kasnije urađena je TE. Za procenu fibroze jetre korišćen je METAVIR skor, a sve preparate biopsija proverio je jedan patolog. APRI skor – odnos aspartat aminotransferaze (AST) i trombocita, Forns indeks, odnos AST i alanin transaminaze (ALT) i TE poređene su sa stepenom fiboze jetre dobijene na osnovu biopsija jetre u cilju dobijanja najboljeg neinvanzivnog markera u proceni fibroze jetre. **Rezultati.** Dokazana je statistička značajnost ($p < 0.05$) za APRI skor, Forns indeks i TE za procenu stepena fibroze jetre. TE je imala najbolji dijagnostički učinak u poređenju sa ostalim markerima koje smo istraživali. Optimalne granične vrednosti za TE bile su 4.25 i 5.9 kPa za dijagnozu fibroze jetre i razlikovanje slabe/umerene i uznapredovale fibroze. Površina ispod krive operativnih

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karakteristka (AUROC) za TE bila je 0.963 i 0.865. **Zaključak.** Na osnovu rezultata ove studije proizilazili su APRI skor, Forns indeks i TE adekvatni dijagnostički markeri fibroze jetre kod bolesnika sa primarnom biijarnom cirozom, ali je TE najsenzitivniji i najspecifičniji parametar. Koristeći neinvanzivne parametre i metode u svakodnevnoj kliničkoj praksi može se smanjiti, ali ne i potpuno izbaciti, potreba za invanzivnim dijagnostičkim procedurama.

Ključne reči:
jetra, ciroza; biopsija; krv, hemijske analize; biološki pokazatelji; elasticitet, tehnike snimanja; osetljivost i specifičnost.

Introduction

Primary biliary cirrhosis (PBC) is a slowly progressing autoimmune disease of the liver that primarily affects middle aged women with an annual incidence ranging from 0.7 to 49 cases per million [1]. Histologically, PBC is characterized by portal inflammation and immune-mediated destruction of intrahepatic bile ducts resulting in further hepatic damage, fibrosis and liver cirrhosis [2].

Liver biopsy remains the gold standard for the liver fibrosis assessment, but it is an invasive and expensive procedure, associated with a low, but negligible risk of complications and mortality [3, 4]. Moreover, the accuracy of liver biopsy in assessing fibrosis has been questioned because of sampling errors as well as intraobserver and interobserver variability [5].

In the last decade, numerous noninvasive methods for the assessment of liver fibrosis were developed and evaluated. Ideally, the test should be reliable, fast, reproducible, easily applicable in every day clinical practice as well as acceptable for patients and reliable for both prognosis and staging of liver disease.

However, most of these methods have been extensively studied in viral hepatitis, but not much has been done regarding patients with PBC [6–9]. The aim of this work was to compare the diagnosis accuracy of liver stiffens – transient elastography (TE) with simple and routinely available blood markers: aspartate aminotransferase (AST) to platelet ratio index (APRI), the Forns index, AST to alanine transaminase (ALT) ratio using liver biopsy as the reference in patients with PBC.

Methods

Patients

This study included 122 prospectively selected patients who were diagnosed with PBC at the Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade from June 2009 to January 2011. The diagnosis of PBC was based on at least 2 out of 3 criteria including elevated serum alkaline phosphatase (ALP), presence of serum antimitochondrial antibodies (AMA) and liver histology consistent with PBC. The diagnosis was confirmed on the basis of the presence of a typical clinical picture, biochemical (elevated ALP ≥ 1.5 times the upper normal value for over 24 weeks) and serological markers (AMA in serum ≥ 1:40) as well as characteristic histological findings on liver biopsy in absence of extrahepatic biliary obstruction. Histological staging was classified ranging from portal tract inflammation with predominantly lymphoplasmacytic infiltrates and septal and interlobar bile duct loss (stage I) to cirrhosis (stage IV). On the same day, each patient underwent blood testing and liver biopsy, while liver stiffness measurements (LSM) using the TE technique were carried out during the following month. Exclusion criteria were presence of ascites, obesity (body mass index > 30 kg/m^2), hepatocellular carcinoma, hepatothropic virus infection, history of alcohol abuse, and all other causes of chronic liver injuries.

Surrogate markers of liver fibrosis

The following serum parameters were examined by venous blood sampling and were processed in our hospital labaratory: AST measured in (IU/L), ALT mesured in IU/L, gamma-glutamyl transpeptidase (GGT) in IU/L, ALP in IU/L, platelets (Pt ×10^9/L), total bilirubin measured in μmol/L, albumins in g/L, cholesterol measured in mmol/L, and prothrombin time (PT – normal range 9.5–13.5 s). On the basis of these biological tests, we calculated the following scores for predicting liver fibrosis: AST/ALT ratio, APRI score = [(AST/upper limit of normal AST) ×100] / number of platelets (10^9/L) [9, 17] and Forns score = 7.811 - 3.131 × ln [number of platelets (10^9/L)] × 0.781 ln [GGT (U/L)] + 3.467 × ln [age (years)] – 0.014 [cholesterol (mg/dL)] [10].

Liver biopsy

Percutaneous liver biopsy was performed on each patient and specimens were routinely processed. Only specimens at least 2 cm long were selected and used for this investigation. Adequate biopsy specimens were obtained from 122 patients. Sections were analyzed independently by a single experienced pathologist unfamiliar with the patients clinical details and results of the noninvasive methods. Liver fibrosis was evaluated semiquantitatively according to the METAVIR scoring system. Fibrosis was scored on a scale of 0–4 as follows: F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis; F4 = cirrhosis. Histological evaluation included grade of inflammation, ductopenia, bile duct inflammation and destruction, cholestasis and ductal proliferation.

Liver stiffness measurement

Liver stiffness was measured by transient elastography using a FibroScan® (EchoSens, Paris, France) equipped with

an M probe. The measurements were obtained from the right lobe of the liver. The patients lay in the dorsal decubitus position with the right arm maximally abducted, through the intercostal spaces between 25 mm and 65 mm from the skin surface. Only examination with 10 valid measurements at a success rate of at least 60% (ratio of the number of successful attempts over the total number of attempts) and an interquartile range less than 30% were considered reliable and kept for statistical analyses. The final result was the median of 10 valid measurements and was expressed in kPa.

Statistical analysis

We used methods of descriptive and analytical statistics. Basic descriptive statistics included means, standard deviations, ranges and percentages. Normal distribution of continuous data was tested using the Kolgomorov-Smirnov test. Analysis of variance (ANOVA) or Kruskal- Wallis test were used for assessment of differences among groups. The diagnostic performance of noninvasive liver assessment methods were performed by receiver operating characteristic (ROC) analysis. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS®, version 14.0).

Ethics

This study was approved by the Ethical Committee of our hospital and all patients signed informed consent before inclusion into the study.

Results

Patients

From the total of 122 patients, 106 (86.5%) of the patients were women with a mean age of 57.40 ± 8.92 years. Their clinical details are summarized in Table 1. All the patients were treated with UDCA (ursodeoxycholic acid) after determining the diagnosis of PBC, 23 (19.2%) patients were without fibrosis (F0), 38 (30.8%) patients had mild fibrosis (F1), 12 (9.6%) patients moderate (F2), 16 (13.5%) patients advanced fibrosis (F3), and 33 (26.9%) patients had liver cirrhosis (F4).

Noninvasive serum parameters

The values of noninvasive serum markers were compared for each histological fibrosis stage (Table 2). We found a difference among APRI, the Forns index and TE

Table 1

Baseline clinical, biochemical and histological characteristics of patients with liver fibrosis (n = 122)

Variables	r ± SD (mediana, min-max)
Age, (years)	57.4 ± 8.9 (58; 42–75)
Alkaline phosphatase (IU/L)	137.9 ± 87.6 (98; 28–351)
Gamma-glutamyl transpeptidase (IU/L)	123.1 ± 142.0 (68; 13–603)
Aspartate aminotransferase (AST), (IU/L)	48.0 ± 30.3 (46; 14–176)
Alanine aminotransferase (ALT), (IU/L)	50.8 ± 27.7 (46; 18–158)
Bilirubin (μmol/L)	13.8 ± 8.3 (11.2; 3.5–36.5)
Platelets (10^9/L)	209.1 ± 87.2 (212; 52–422)
Albumine (g/L)	39.5 ± 4.6 (40; 28–51)
Protrombin time (PT), s	90.2 ± 18.2 (93; 43–125)
Cholesterol (mmol/L)	5.1 ± 1.4 (5.43; 2.33–8.73)
AST/ALT	0.9 ± 0.3 (0.97; 0.41–1.70)
AST platelet ratio index	0.6 ± 0.7 (0.40; 0.10–2.60)
Forns index	6.0 ± 2.1 (5.47; 2.75–10.85)
Transient elastography (kPa)	9.6 ± 6.9 (6.8; 3.2–30.7)
METAVIR scoring system, n (%)	
F0	23 (19.2%)
F1	38 (30.8%)
F2	12 (9.6%)
F3	16 (13.5%)
F4	33 (26.9%)

r ± SE – mean ± standard deviation; n (%) – number (percentage) of patients.

Table 2

Surrogate markers of liver fibrosis

Variables	METAVIR Score (r ± SD)				
	F0 n = 23	F1 n = 38	F2 n = 12	F3 n = 16	F4 n = 33
AST/ALT[a] (IU/L)	0.9 ± 0.2	1.0 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	1.0 ± 0.3
APRI*[b] (IU/L/10^9)	0.2 ± 0.1	0.5 ± 0.6	0.4 ± 0.2	0.9 ± 0.9	1.0 ± 0.7
Forns index*[a]	4.3 ± 0.9	5.8 ± 2.0	5.4 ± 0.5	7.0 ± 3.0	7.4 ± 2.4
TE (kPa) *[b]	5.5 ± 0.2	6.4 ± 2.1	9.3 ± 4.3	10.8 ± 3.9	17.7 ± 7.8

***Statistically significant differences; [a]One way ANOVA; [b]Kruskal -Wallis test; AST – aspartate aminotransferase; ALT – alanine aminotransferase; APRI – AST platelets index; TE – transient elastography.**

according to stages of liver fibrosis. AST/ALT ratio did not show any significant difference. For these parameters, we calculated sensitivity, specificity and Area under the receiver operating characteristic (AUROC), as presented in Table 3 and Figure 1, as well as best cut-off values for determining the presence of liver fibrosis (Table 4 and Figure 2). A cut-off value of 0.255 for the APRI score as well as the Forns index cut-off value of 4.168 were statistically significant for predicting existence of liver fibrosis. Also, we recognized a statistically significant possibility for distinguishing patients having mild-to-moderate fibrosis (F1 or F2) and advanced fibrosis (F3 or F4) as presented in Tables 5 and 6, and Figures 3 and 4, respectively.

Table 3

Area under the reciever operating characteristic (AUROC) curve for surrogate markers of liver fibrosis (fibrosis: no vs. yes)

Surrogate markers of liver fibrosis	AUROC	Asymp significance	95% Confidence interval (bound: lower-upper)
AST/ALT (IU/L)	0.588	0.390	0.419–0.758
APRI (IU/L/10^9)	0.782	0.006*	0.649–0.915
Forns index	0.806	0.003*	0.685–0.927
TE (kPa)	0.963	0.000*	0.000–1.000

***Statistically significant; AST – aspartate aminotransferase; ALT – alanine aminotransferase; APRI – AST-platelet index; TE – transient elastography.**

Table 4

Sensitivity and specifity for surrogate markers of liver fibrosis (fibrosis: no vs. yes)

Surrogate markers of liver fibrosis	Cut-off	Sensitivity	Specifity	Asymp significance	AUC (95% CI)
AST/ALT	0.917	0.571	0.600	0.403	0.586 (0.388–0.783)
APRI	0.255	0.810	0.600	0.046*	0.705 (0.510–0.899)
Forns index	4.168	0.881	0.500	0.063	0.690 (0.486–0.894)
TE (kPa)	7.250	0.929	1.000	0.000*	0.964 (0.001–0.999)

AUC – area under the curve; for other abbreviations see Table 3;*statistically significant.

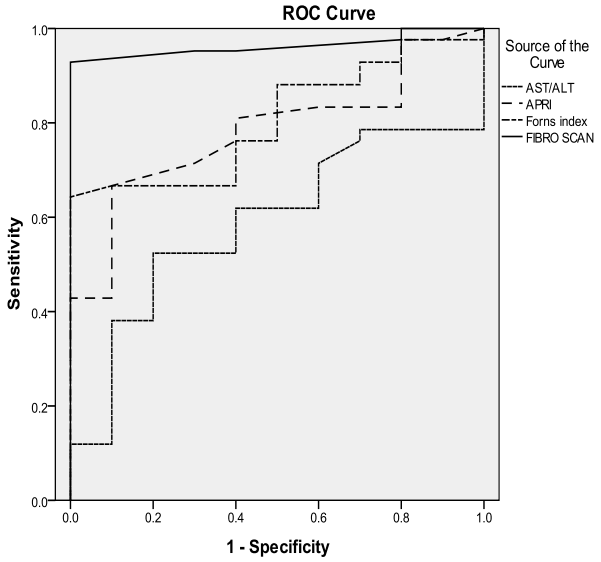

Fig. 1 – ROC curve for surrogate markers of liver fibrosis. ROC – receiver operating characteristic. For abbreviations see Table 3.

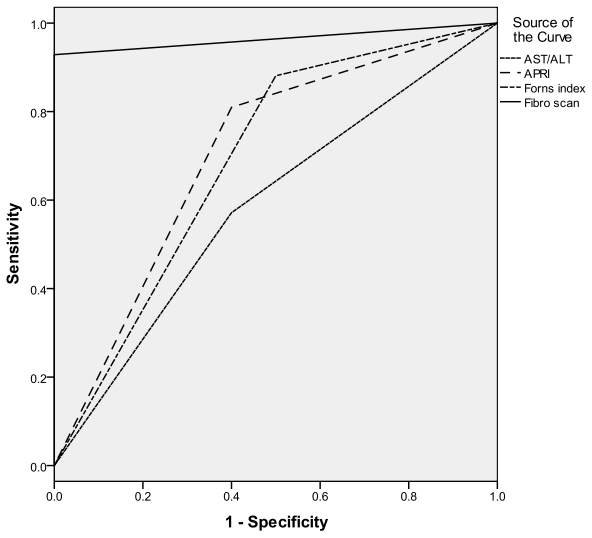

Fig. 2 – ROC curve for surrogate markers of liver fibrosis with cut-off. For abbreviations see Table 3.

Table 5

Area under the curve (AUC) for surrogate markers of liver fibrosis (fibrosis: stage I–II vs. III–IV)

Surrogate markers of liver fibrosis	AUC	Asymp significance	95% Confidence interval (bound: lower-upper)
AST/ALT	0.428	0.480	0.235–0.621
APRI	0.601	0.323	0.396–0.806
Forns index	0.676	0.084	0.495–0.858
TE (kPa)	0.865	0.000*	0.747–0.984

For other abbreviations see Table 3;*statistically significant.

Table 6

Sensitivity and specifity for surrogate markers of liver fibrosis (fibrosis: stage F – I-II vs. F – III-IV)

Surrogate markers of liver fibrosis	Cut-off	Sensitivity	Specifity	Asymp significance	AUC (95% CI)
AST/ALT	1.010	0.500	0.615	0.572	0.558 (0.358–0.757)
APRI	0.297	0.667	0.577	0.233	0.622 (0.429–0.814)
Forns index	5.460	0.750	0.654	0.048*	0.702 (0.522–0.882)
TE (kPa)	9.900	0.917	0.692	0.003*	0.804 (0.659–0.950)

AUC – area under the curve; for other abbreviations see Table 3; *statistically significant.

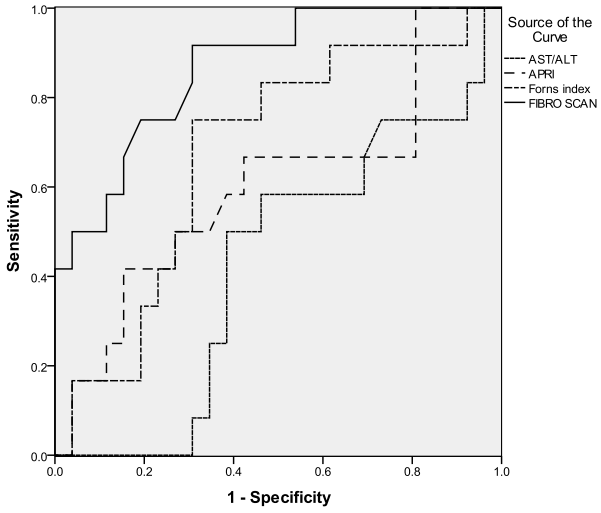

Fig. 3 – ROC for surrogate markers of liver fibrosis, fibrosis: stage F – I-II vs. F – III-IV. For abbreviations see Table 3.

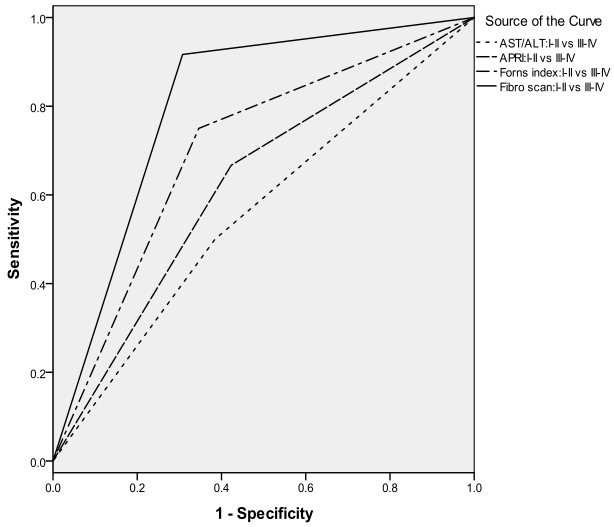

Fig. 4 – ROC curve for surrogate markers of liver fibrosis: stage F – I-II vs. F – III-IV with cut-off. For abbreviations see Table 3.

Transient elastography findings

The values of TE ranged from 3.2 to 30.7 kPa, median 6.8 kPa, mean 9.6 ± 7.0 kPa. A statistically significant difference was found between TE and fibrosis stage of liver disease (Tables 2 and 3). TE was found to be accurate in diagnosing the presence of liver fibrosis. For cut-off value of 7.25 kPa, it showed 92.9% of sensitivity and 100% of specificity (AUROC 0.964). These results were the best when compared to other surrogate markers of fibrosis. The optimal cut-off values for distinguishing patients having mild-to-moderate fibrosis (F1 or F2) and advanced fibrosis (F3 or F4) were 9.9, with 91.7% of sensitivity and 69.2% of specificity and AUROC 0.865 (Tables 5 and 6, and Figures 3 and 4, respectively). These results were superior in comparison to other surrogate markers for liver fibrosis.

Discussion

Primary biliary cirrhosis occurs worldwide with a female to male ratio of 9:1. The diagnosis of PBC is based on criteria which include elevation of liver enzymes, positive AMA test and positive liver biopsy. Widespread use of screening laboratory tests has led to an increase of PBC diagnosis frequency while the disease is in asymptomatic stage. Liver biopsy has been considered to be a gold standard for the diagnosis of PBC, even though fibrosis in PBC is patchy in distribution within the parenchyma and many patients are reluctant to experience repeated biopsies which limits our ability to monitor disease progression and effects of treatment.

In recent decades, a lot has been done in order to find adequate noninvasive markers for the assessment of liver fibrosis. The ideal characteristics of such markers would be: specificity for liver fibrosis; providing measurement of: stage of fibrosis, fibrogenesis activity; not influenced by comorbidities (e.g. renal, reticulo-endothelial); known half-life; known excretion route; sensitivity and reproduciblity [11]. Direct markers are markers of fibrogenesis, measurable in peripheral blood as a direct expression of either the deposition or removal of ECM in liver (several glycoproteins, the collagen family, the collagenases and their inhibitors and a number of cytokines connected with the fibrogenetic process). Indirect markers of liver fibrosis are routinely performed blood tests. The diagnostic performance of most direct and indirect markers of liver fibrosis has been widely investigated in all common etiological forms of chronic liver diseases, but not as much in patients with PBC. Unfortunately, there are currently no serum surrogate markers of liver fibrosis routinely recommended in PBC.

Our study is the PBC specific and it was conducted on particulary homogenous study population recruited from a single center. On the same day, each patient involved in this

study, underwent blood tests and liver biopsy, while TE was performed in the following month.

In this study we investigeted 3 noninvasive markers, available and routinely used in every day clinical practice. The AST/ALT ratio, although widely used, did not show any statistical significance.

As far as we know, there are few published studies assessing the APRI score in the PBC patients [7, 9, 11, 12]. Obara et al. [13] showed that the APRI score can predict fibrosis F ≥ 2 (AUROC O.77) in patients with nonviral liver diseases. In our study that was not the case. The APRI score did not show statistically significant difference in distinguishing patients with mild-to-moderate fibrosis and advanced fibrosis, perhaps because the study population was not the same. In our study, the APRI score showed statistically significant difference in presence and stages of liver fibrosis (AUROC 0.782). A cut-off value of 0.255 (sensitivity 81%, specificity 60%, AUROC 0.705) could distinguish patients who did not have (F0) and those who had liver fibrosis (F1).

In our study, the Forns index, although not so widely used and investigated in cholestatic liver disease, did correlate with the presence and stages of liver fibrosis (AUROC 0.806). The optimal cut-off for F ≥ 3 was 5.46 (sensitivity 75%, specificity 65.4%, AUROC 0.702).

In 2012, a retrospective study was conducted in China [14]. It included 73 patients with PBC and assessed the diagnostic value of noninvasive markers of liver fibrosis in PBC based on coventional laboratory results (platelet count, serum cholinesterase, albumin, HDL-C and prothrombin time activity). According to this study, the established noninvasive model could accurately distinguish pathological changes of early stage of PBC (stage I–II) from advanced stage (III–IV).

TE is a novel, noninvasive method used for evaluation of fibrosis in chronic liver disease. Published meta analyses have shown that TE is a reliable method for diagnosing liver cirrhosis [15–17]. The effectiveness of TE is well established in patients with chronic hepatitis C [18], but it has not been used widely in assessing fibrosis in nonviral liver disease. In the past few years, a very small number of studies investigating the effectiveness of TE in evaluation of patients with PBC was published.

A study conducted in Spain including 80 patients with PBC, showed statistically significant correlation between TE and liver biopsies [6], while another study from Italy conducted on 120 patients with PBC proved that TE is a simple, reliable and useful method for assessing liver fibrosis [7].

Coprechot et al. [8] assessed 140 patients with PBC in order to define the diagnostic performance of TE and the time course of changes of fibrosis progression as well as prognosis in a monitored cohort of ursodeoxycholic acid (UDCA)–treated patients followed up for five years. Their results showed that TE is one of the best current surrogate markers of liver fibrosis in PBC. In a five year period while on the treatment, liver stiffness appeared to remain stable in most noncirrhotic patients, whereas it significantly increased in patients with cirrhosis. The study did not find evidence that combination of TE and noninvasive markers significantly improved diagnostic accuracy.

Present findings strongly suggest that monitoring of TE in patients with PBC provides significant prognostic information in comparison with classic serum prognostic markers and that it may be used to predict outcome and select high-risk patients for further clinical trials [8].

In addition, TE efficiency was validated by comparing it to other imaging techniques. Friedrich-Rust et al. [9] compared TE, magnetic resonance imaging (MRI) and spectroscopy MRI, and serum markers in 45 PBC patients. They showed that MRI and TE can be used with comparable results for the assessment of liver fibrosis in patients with PBC and that the two techniques seem to supplement each other.

In our study, TE appears to be the best surrogate marker for assessment of liver fibrosis in patients with PBC. When compared to other noninvasive liver markers, TE was shown to have the highest sensitivity and specificity.

Conclusion

Assessment of liver fibrosis by TE is an easy, rapid, effective, and safe noninvasive method with high sensitivity and specificity. Using noninvasive markers and methods in evaluating patients in daily clinical practice may reduce, but still not eliminate, the need for invasive diagnostic procedures.

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