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Validation of Biochemical Markers for the Prediction of Liver Fibrosis and Necroinflammatory Activity in Hemodialysis Patients with Chronic Hepatitis C

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Key Words

Hemodialysis · Chronic hepatitis C · FibroTest-ActiTest · Fibrosis · Necroinflammatory activity · METAVIR scoring · AST-to-platelet ratio index

Abstract

Background: Liver biopsy is an imperfect gold standard for assessing the disease severity in hemodialysis patients with chronic hepatitis C. Our purpose was to compare the accuracy of the FibroTest (FT) and ActiTest (AT) with liver biopsy and the AST-to-platelet ratio index (APRI) in determining hepatic fibrosis and necroinflammatory activity in hemodialysis patients with hepatitis C virus (HCV). ***Methods:*** The FT-AT index combining 6 biochemical markers was assessed in 33 hemodialysis patients with HCV. Liver fibrosis and necroinflammatory activity was staged and graded according to the METAVIR scoring system. ***Results:*** The accuracy of FT-AT versus biopsy was 0.46 for significant fibrosis and 0.36 for severe necroinflammatory activity. The FT index had a positive predictive value of 20% for scores greater than 0.6 and a negative predictive value of 45% for scores less than 0.2. Eleven of the 33 patients had scores ≤0.2, 6 had significant fibrosis on biopsy. Four out of 5 patients with FT scores >0.6 had mild fibrosis. APRI correlated well with the biopsy. ***Conclusion:*** The FT-AT test does not seem to be a reliable noninvasive marker for the prediction of necroinflammatory activity and fibrosis in hemodialysis patients with HCV and cannot be used as an alternative to either liver biopsy or APRI.

Introduction

Hemodialysis patients are at high risk of acquiring hepatitis C virus (HCV) and the prevalence of hepatitis C is high in this population (up to 60%) [1, 2]. Considering the risk of interferon-induced graft rejection and cirrhosis which is a contraindication for kidney transplantation, interferon therapy should be recommended to dialysis patients at the diagnosis very early and prior to transplantation surgery [3, 4]. Liver biopsy is mandatory for the

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management of patients with HCV. This procedure is recommended before the initiation of antiviral therapy [5–7] and is vital for monitoring the progression of fibrosis. Unfortunately, liver biopsy is invasive, costly and associated with certain complications such as hemorrhage, especially in patients with congenital or acquired disorders of coagulation. Considering the hemorrhagic risk in hemodialysis patients and patients' reluctance, noninvasive predictors of histology have been studied [8]. The FibroTest (FT) and ActiTest (AT) are widely used as a noninvasive alternative to liver biopsy in patients with HCV and include combinations of simple serum markers of fibrosis and necroinflammatory activity. However, several questions persist concerning the discrepancies between FT/AT scores and histopathological findings. Therefore, the aim of this study was to assess the diagnostic value of FT and AT in comparison with liver histology in hemodialysis patients with HCV infection.

Patients and Methods

Thirty-three patients with chronic HCV, who were undergoing intermittent hemodialysis 3 times a week, 4 h on each session, were enrolled into the study. Chronic hepatitis C was defined as having a positive serologic test for HCV and a compatible liver biopsy. The high prevalence of HCV infection in hemodialysis patients is believed to be attributed to the transfusion of HCV-contaminated blood products or to nosocomial transmission of HCV within hemodialysis units. Therefore, we assumed the date of contamination to be the date of the first blood transfusion or the first hemodialysis. Anti-HCV and HCV RNA positivity for at least 6 months were required. To be eligible, patients had to have a serum sample which was drawn within 1 week before or after the liver biopsy and stored at –80°C. Patients were excluded if they had a noninterpretable biopsy, complicated cirrhosis, positive HBsAg serology, HIV infection, iron overload, Wilson's disease and a history of alcohol consumption more than 20 g/day. Anyone on hepatotoxic drug, immunosuppressant or putative antifibrotic treatment was excluded from the study. The study protocol was approved by a local ethics committee according to the Declaration of Helsinki.

FT-AT indexes were determined by a panel of 5 biochemical markers: α2-macroglobulin, apoliprotein A1, haptoglobin, γ-glutamyltranspeptidase (γ-GT), and total bilirubin for liver fibrosis (FT) and alanine aminotransferase (ALT) for liver necroinflammatory activity (AT) [9]. We calculated a novel index, another noninvasive fibrosis marker, termed the AST (aspartate aminotransferase)-to-platelet ratio index (APRI) as follows: APRI = (AST level/upper limit of normal)/platelet counts (10^9/liter) × 100 [10].

Immunonephelometric Methods

Assays of α2-macroglobulin, haptoglobin and apolipoprotein A1 were performed on BN2 or BN ProSpec analyzers (Dade Behring, Malburg, Germany).

Immunoturbidimetric Methods

Assays of haptoglobin and apolipoprotein A1 were performed on an Integra 400 analyzer and reagents marketed by Roche Diagnostics GmbH (Mannheim, Germany). ALT and γ-GT enzymatic activity measurements were performed at 37°C and calibrated with CFAS (calibrator for automated systems, Roche Diagnostic Society). Szasz enzymatic method standardized against the original Szasz method [11] and IFCC (International Federation of Clinical Chemistry) standardized enzymatic method [12] were used for γ-GT and ALT, respectively.

The FT/AT provides a numerical quantitative estimate ranging from 0.00 to 1.00 corresponding to the METAVIR scoring system of fibrosis stages F0–F4 and of necroinflammatory grades A0–A3. Corresponding stages and necroinflammatory grades were calculated by median scores and 95% CI. FT/AT indexes were estimated using the biopredictive website by an investigator who was unaware of the results of the liver biopsy.

Serum HCV-RNA testing was performed at a single central laboratory by a quantitative polymerase chain reaction with a detection limit of 100 copies/ml [13]. HCV genotyping was performed using INNOLIPA HCV as previously described [14].

Biopsy specimens were fixed in a formalin-alcohol-acetic acid solution. Paraffin-embedded 5-μm thick sections were stained with hematoxylin-eosin. The histological features were analyzed by the same pathologist (G.O.) according to the METAVIR scoring system [15]. Fibrosis was staged on a scale from F0 to F4: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis. Necroinflammatory activity was graded as follows: A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity. Biopsies were performed using Menghini's technique with a 1.4-mm diameter Hepafix Luer Lock needle (Braun Melsungen).

Statistical Analysis

Continuous variables were presented as means ± SD, and groups were compared using Student's t and Mann-Whitney tests. Categorical variables were compared by the χ^2 and Fisher's exact tests. Diagnostic value was assessed by the area under the receiver operating characteristic curve (AUROC). The cutoff levels were defined as the test values giving the best sensitivity and specificity. The diagnostic accuracy was calculated by positive and negative predictive values (PPV and NPV). Multiple regression analysis was used for multivariate analysis to identify the independent variables for the FT score severity. Binary logistic regression with the inclusion of associated factors with significant fibrosis was used. All data were analyzed using SPSS statistical software (Ver.10.0). A p value <0.05 was considered statistically significant.

Results

A total of 33 consecutive, previously untreated patients (m/f: 18/15; mean age ± SD: 37 ± 10.63 years) with serologically confirmed chronic HCV infection were evaluated. Demographic and biochemical data, necroinflammatory activities and fibrosis stages estimated by the FT/AT are presented in table 1. Most of the patients were in-

Table 1. Demographic, histologic and biochemical parameters of the 33 HCV-infected hemodialysis patients

Baseline characteristics	Value
Demographics	
Age, years	37 ± 10.63
Male	18 (54.5)
Determination of genotype	
1a	3 (9.1)
1b	25 (75.7)
2a	2 (6.1)
3a	3 (9.1)
Histology estimated by biopsy	
Bridging fibrosis F2–F4	16 (48.5)
Moderate-severe activity A2–A3	21 (63.6)
Histology estimated by biochemical markers	
FT score (0.00–1.00)	0.34 ± 0.22
Bridging fibrosis F2–F4	21 (63.6)
AT score (0.00–1.00)	0.13 ± 0.18
Moderate-severe necroinflammatory activity A2–A3	7 (21.2)
Biochemistry (normal range)	
ALT, IU/l (female 0–40; male 0–48)	36.90 ± 86.56
γ-GT, IU/l (female 5–36; male 8–61)	51.60 ± 51.10
Total bilirubin, μmol/l (5.13–20.52)	9.82 ± 4.20
α2-Macroglobulin, g/l (female 1.6–4.0; male 1.4–3.3)	1.95 ± 0.57
Haptoglobin, g/l (0.4–2.4)	0.87 ± 0.58
Apoliprotein A1, g/l (1.2–1.7)	1.11 ± 0.16

Data are presented as means ± SD or proportions [n (%)].

Table 2. Factors associated with the severity of FT score

Parameter	β	p
γ-GT, IU/l (female 5–36; male 8–61)	0.001	0.001
Total bilirubin, μmol/l (5.13–20.52)	0.005	0.01
α2-Macroglobulin, g/l (female 1.6–4.0; male 1.4–3.3)	0.18	0.0001
Haptoglobin, g/l (0.4–2.4)	–0.17	0.0001
Apoliprotein A1, g/l (1.2–1.7)	–0.20	0.049

fected with genotype 1b (75.7%). The mean duration of dialysis was 5.95 ± 4.29 years. The leading causes of renal failure were chronic glomerulonephritis (6%), diabetes mellitus (6%), polycystic kidney disease (6%), interstitial nephritis (6%), hypertensive nephrosclerosis (6%), systemic lupus erythematosus (3%), Still's disease (3%) and shrunken kidney with unknown etiology (64%). The mean duration after the diagnosis of HCV was 5.95 ± 4.29 years. We found no statistical confirmation among this parameter and other studied parameters.

Liver Histology

The mean size of biopsy specimens was 16 ± 4 mm. Biopsies with ≥5 portal tracts were obtained. The histological fibrosis results were as follows: 7/33 (21.21%) had no fibrosis, 10/33 (30.3%) minimal fibrosis (F1), 11/33 (33.33%) mild fibrosis (F2), and 5/33 (15.15%) severe fibrosis (F3). None of the patients had cirrhosis. Proportions of the necroinflammatory scores for A0, A1, A2 and A3 were 4/33 (12.12%), 8/33 (24.24%), 12/33 (36.36%) and 9/33 (27.27%) patients, respectively.

Factors Associated with the Degree of Fibrosis

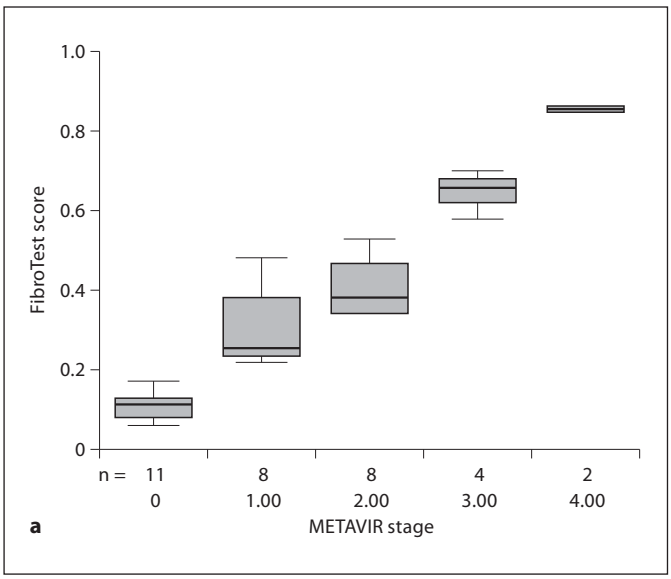
Multiple logistic regression analysis was performed in order to determine whether the biochemical parameters were predictive of advanced fibrosis. By multivariate analysis, including the components of the FT index, γ-GT, bilirubin, α2-macroglobulin, haptoglobin and apolipoprotein A1 were independently associated with the severity of the FT score (table 2). But the FT score was not associated with the presence of significant fibrosis (OR: 1.46, p = 0.8, CI: 0.07–30.9).

Discordance between FT/AT Indexes and METAVIR Scores

Figure 1 depicts the box plots illustrating the relationship between FT/AT indexes and the fibrosis stages and necroinflammatory grades assessed using the METAVIR classification. Considering a greater than or equal to 2 point difference, the discordance rate was 9.1% (3/33) for necroinflammatory activity (1 overestimation and 2 underestimations by the AT compared with biopsy) and 24.8% (8/33) for fibrosis (8 overestimations by the FT compared with biopsy). An exact correlation between FT/AT scores and biopsy was observed in 14/33 (42.42%; necroinflammatory activity), and in 11/33 (33.33%; fibrosis score).

Validation of Serum FT/AT Indexes

The diagnostic values of the FT and AT indexes to estimate the fibrosis stage and the necroinflammatory activity were assessed by the AUROC analysis as shown in figures 2 and 3. The ROC curve used to establish the discriminative power of the FT index for fibrosis severity did not show any significant sensitivity and specificity (fig. 2). The FT threshold for the prediction of significant fibrosis (F2–F4) was 0.19; at this threshold, the sensitivity and



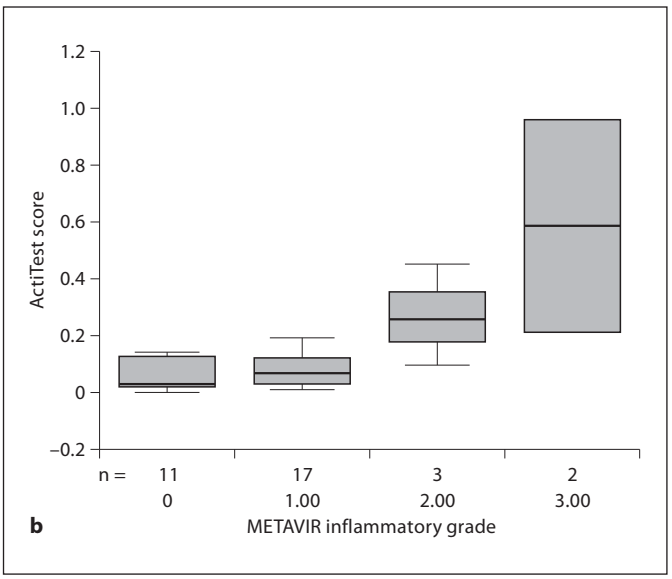


Fig. 1. Box plots of biochemical FT (**a**) and AT (**b**) indexes according to the corresponding METAVIR fibrosis stage and necroinflammatory grade. The top and the bottom of the box are the 25th and 75th percentiles. The line through the box is the median. The vertical lines from the ends of each box encompass the extreme data points.

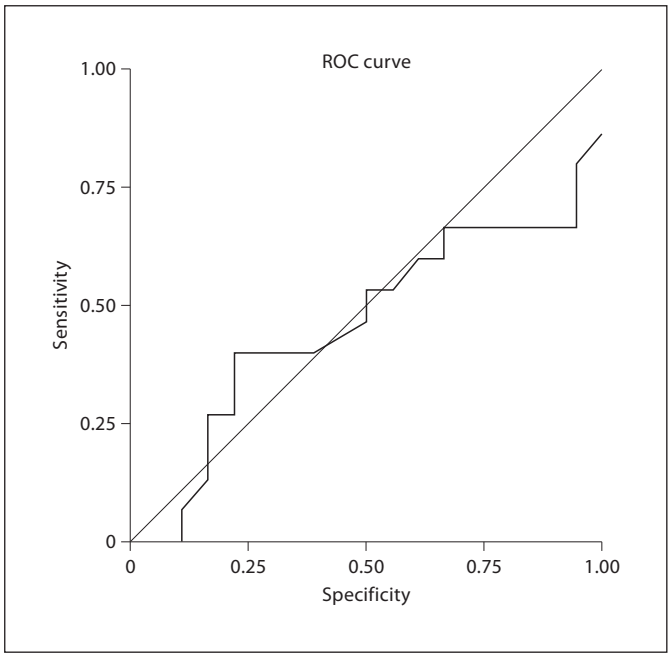


Fig. 2. ROC curve for the prediction of the discriminative power of the FT index for fibrosis severity (AUROC: 0.46; 95% CI: 0.25–0.67).

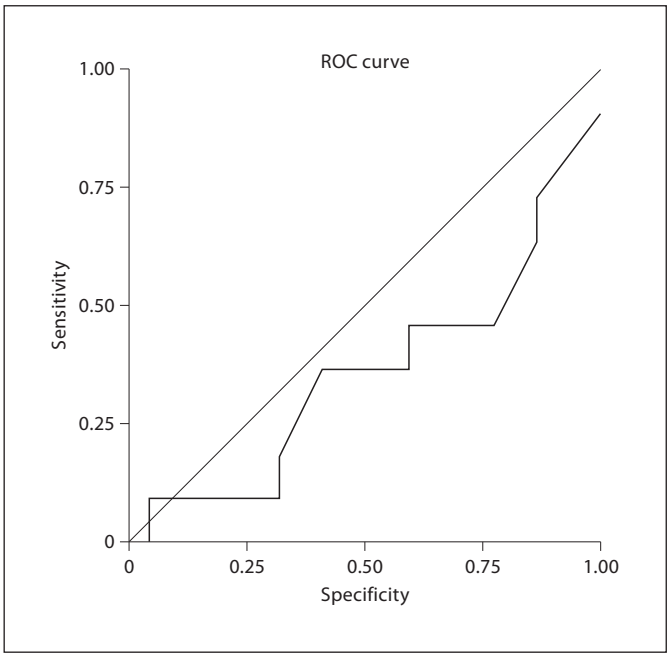


Fig. 3. ROC curve for the prediction of necroinflammatory activity using the AT index (AUROC: 0.36; 95% CI: 0.15–0.57).

specificity was 64% and 68%, respectively. The AUROC was 0.46. The AUROC for the diagnosis of necroinflammatory grade, A0–A1 versus A2–A3, was 0.36 (fig. 3). The best cutoff was at 0.11 with a sensitivity of 36% and a specificity of 40%.

If the cutoff values of the previously published FT scores ≤0.2 with a high NPV for advanced fibrosis (certainty of absence of significant or severe fibrosis) and values >0.6 with a PPV (certainty of presence of significant or severe fibrosis) were used [8, 16], the NPV of a score ≤0.2 would be 45.45% (5/11) and the PPV of a score >0.6 would be 20% (1/5) in our study. At a threshold of >0.6, the sensitivity and specificity for the presence of significant fibrosis (F2–F4) were only 13 and 16%, respectively.

The stage of fibrosis was estimated correctly in 11 (33.33%) patients. Three (9.1% of all) out of 5 patients with a score <0.1, and 5 (15.15% of all) of 11 patients with a score ≤0.2 were deemed unlikely to have significant fibrosis. One (3% of all) out of 5 patients with a score >0.6 (likely to have significant fibrosis) did not require liver biopsy. The NPV of a score <0.1 was 60% (3/5). Of the 11 patients with a FT score ≤0.2, a false-negative result, significant fibrosis was observed in 6 (18.18% of all patients) patients. Treatable fibrosis would have been missed in these 6 patients. Of the 5 patients with a FT score >0.6, a false-positive result, absence of significant fibrosis was observed in 4 (12.12% of all) patients. These 4 patients with nonsignificant fibrosis, who had not required antiviral therapy according to consensus recommendations, would have been treated.

In bivariate analysis, we found that the APRI correlated significantly with the stage ($r = 0.47$, $p = 0.02$). The multivariate analysis also revealed that the APRI was independently associated with the severity of fibrosis ($\beta = 0.23$, $p = 0.02$). In bivariate analysis, histopathological findings-necroinflammatory activity grade and fibrosis did not correlate with HCV-RNA levels ($r = 0.48$, $p = 0.16$, and $r = 0.04$, $p = 0.92$, respectively). The multivariate analysis revealed that the necroinflammatory grade and the severity of fibrosis were independent from the effect caused by increased HCV-RNA levels ($\beta = 0.23$, $p = 0.16$, and $\beta = 0.14$, $p = 0.92$, respectively).

Discussion

Hemodialysis patients belong to a distinct group in which the prevalence of chronic HCV infection is high, ranging from 10 to 65% [2, 16, 17]. Morbidity and mortality in chronic hepatitis C patients depend on the rate of development of liver fibrosis. Accurate staging of HCV-related fibrosis is crucial for the treatment decision and prediction of prognosis. Although liver biopsy is acknowledged as the gold standard in the evaluation of fibrosis, it is invasive and associated with complications including hemorrhage and death in 0.3–0.6% of the patients [18–20]. These risks may be enhanced in dialysis patients due to hemostatic abnormalities [21].

Because of the associated risks, the need for hospital stay and the cost of liver biopsy are substantially higher in dialysis patients. The noninvasive prediction of HCV-related liver injury has gained wide acceptance. Therefore, we assessed the diagnostic accuracy of the biochemical markers of liver fibrosis (FT) and necroinflammatory activity (AT) as an alternative to liver biopsy in 33 hemodialysis patients with HCV. 33.33% of patients were correctly classified using the FT and AT, but our results were not consistent with earlier studies. The values were generally smaller than those of the general population.

In a systematic review and a prospective multicenter study, the diagnostic value of the FT and AT in patients with chronic HCV infection was confirmed and the AUROC for the diagnosis of moderate-to-severe necroinflammatory activity was reported to be 0.73 [22, 23]. This was not different from the other validation studies [9, 24, 25]. In the present study, the AT was only associated moderately with necroinflammatory activity. The AUROC for the diagnosis of necroinflammatory grade for A0–A1 versus A2–A3 was 0.36. The FT is the most studied biomarker in more than 30 different populations. The mean AUROC for the diagnosis of advanced fibrosis was 0.84 [26]. The use of FT was recommended by the French Superior Health Authority for the diagnosis of cirrhosis in patients with untreated HCV infection [27].

Investigators from France reported that the FT score in HCV patients had a high NPV (100% certainty of absence of F2–F4) for scores <0.1, and a high PPV (>90% certainty of presence of F2-F4) for scores >0.6 [28]. In a cross-sectional analysis of HCV and HIV/HCV co-infected patients, the NPV of a FT score <0.1 was 0.85 [28, 29]. In our study, the accuracy of an absence of significant fibrosis (F2–F4) was only 60% with a score <0.1 and the accuracy of the presence of significant fibrosis was 20% with a score >0.6. A possible reason for the lower diagnostic accuracy may be due to variations in biochemical components of the FT/AT scores among patients with renal failure.

Abnormalities in lipoprotein metabolism are common in uremic patients. Some observations suggest that a component of uremic serum inhibits hepatic apolipoprotein

A-1 synthesis [30]. α2-Macroglobulin belongs to the acute phase proteins and is produced at sites of inflammation and liver fibrosis [31]. Haptoglobin decreases when fibrosis increases. This may be a response to the increased hepatocyte growth factor seen in liver damage [32]. The inverse correlations between fibrosis and α2-macroglobulin (positive) and haptoglobin (negative) can be explained by acute phase reaction induced by the dialysis procedure [33, 34]. Data in the literature concerning the relationship between the FT accuracy and hemodialysis are very scarce. In the original report, the FT diagnostic accuracy in dialysis patients was found to be similar to that in the general population (75%) [8]. In our study, the PPV of a FT score >0.6 for the presence of significant fibrosis (F2–F4) was 20%, the NPV of a FT score ≤0.2 for the absence of significant fibrosis was 45.45%. Superior data were obtained in the original study in which a PPV of a score >0.6 for the presence of significant fibrosis was 71%, and the NPV of a score ≤0.2 for excluding significant fibrosis was 77%, respectively [2]. The FT/AT enabled concordant classification with necroinflammatory activity in 42.42% and with fibrosis in 33% of patients. The discrepancy resulted from an under- or overestimation of necroinflammatory activity or an overestimation of fibrosis. The overestimation was predominantly influenced by the elevated levels of α2-macroglobulin.

In independent studies, the FT has led to a 46% reduction in liver biopsies [28, 29]. Diagnostic accuracy was around 80% and discrepant results were obtained in 19% of patients [28]. Despite the weaker diagnostic accuracy, the results obtained in the study of Varaut et al. [8] were almost consistent with those above-mentioned earlier studies. 75% of hemodialysis patients were correctly classified by using the FT, and its use meant that 32% of liver biopsies could be avoided. Compared with this original report, we obtained significantly lower values. Liver biopsy could have been avoided in 5 (15.15% of all) of 11 patients with FT scores ≤0.2, and 1 (3% of all) out of 5 patients with FT scores >0.6. Fibrosis was correctly classified in 11 (33.3% of all) of all patients. Liver biopsy remained mandatory for the evaluation of fibrosis in the remaining 22 patients. Various variables that formulate the FT-AT scores may be influenced by nonhepatic conditions such as hematoma and immunological factors which are frequently seen in the hemodialysis population. These conditions may result from high bilirubin and α2-macroglobulin levels and low haptoglobin and apolipoprotein A1 levels [35]. The discrepancy between these 2 studies is probably due to various frequencies of such conditions in study populations.

Some of the markers, such as macroglobulin, haptoglobin and apolipoprotein A1, are not routinely used in clinical practice. With use of more easily available variables, Wai et al. [10] developed the APRI as a noninvasive predictor of fibrosis. The stage of fibrosis has been correctly estimated in 81% of the patients with chronic hepatitis C. We found that APRI correlated significantly with the fibrosis stage and was independently associated with the severity of fibrosis.

The small sample size of HCV-infected hemodialysis patients who underwent liver biopsy was a major limitation of the study. Sample size had to be limited due to safety issues of liver biopsy in hemodialysis patients. The low number of study participants may lead to type II error and false-negative results. The post-hoc power of our study was 74%. This means that our study was underpowered. This should be considered as a pilot study which warrants confirmation by larger studies.

In conclusion, the use of FT-AT with cutoff limits of ≤0.2 and >0.6 would have avoided the liver biopsy in 16 patients, but treatable fibrosis would have been missed in 6 (18.18% of all) of these patients. Considering the possibility of liver complications, it may not be deleterious to delay the initial diagnosis of significant fibrosis. But postponing it until a more advanced stage or cirrhosis can lead to contraindication to kidney transplantation. Therefore, FT-AT could not reliably be used to reduce the need for liver biopsy for the management of chronic HCV infection in hemodialysis patients.

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