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ORIGINAL ARTICLE

Usefulness of acoustic radiation force impulse and fibrotest in liver fibrosis assessment after liver transplant

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ABSTRACT

Background and rationale. Acoustic radiation force impulse (ARFI) is a non-invasive tool used in the evaluation of liver fibrosis in HCV positive immune-competent patients. This study aimed to assess the accuracy of ARFI in discriminating liver transplanted patients with different graft fibrosis severity and to verify whether ARFI, eventually combined with non-invasive biochemical tests, could spare liver biopsies. This prospective study included 51 HCV positive liver transplanted patients who consecutively underwent to annual liver biopsy concomitantly with ARFI and blood chemistry tests measurements needed to calculate several non-invasive liver fibrosis tests. **Results.** Overall ARFI showed an AUC of 0.885 in discriminating between patients without or with significant fibrosis (Ishak score 0-2*vs.* 3-6). Using a cut-off of 1.365 m/s, ARFI possesses a negative predictive value of 100% in identifying patients without significant fibrosis. AUC for Fibrotest was 0.848 in discriminating patients with Ishak fibrosis score 0-2 *vs.* 3-6. The combined assessment of ARFI and Fibro-test did not improve the results obtained by ARFI alone. **Conclusion.** ARFI measurement in HCV positive liver transplanted patients can be considered an easy and accurate non-invasive tool in identify patients with a benign course of HCV recurrence.

Key words. Non-invasive liver fibrosis test. ARFI. HCV. Cirrhosis. Fibrotest.

INTRODUCTION

Recurrent hepatitis C (HCV) after liver transplantation (LT) is universal in recipients who underwent transplant during active HCV replication.[1] About 30% of liver transplanted patients develop graft cirrhosis 5 years after transplant and this represents the more frequent cause of liver related death or re-transplantation.[2] Several factors are responsible for a more rapid course of liver fibrosis progression in liver transplanted patients compared to immune-competent. Established risk factors for disease progression in this patient population include a high donor age, a high HCV viral load before or directly after LT, a human leukocyte antigen mismatch and the occurrence of severe episodes of rejection.[3]

Fibrosis progression of the graft often is not linear and can have an early exponential increase as well as a late start.[4] This indicates that disease activity is variable over time and that current time at a given stage rather than the prior time in earlier stages is most predictive of future progression.[5] The discrimination between patients with slow and rapid fibrosis progression would avoid starting expensive antiviral therapy in those with an expected good long term survival, while using early treatment in those at high risk of disease progression. In fact, it is largely accepted that the optimal time to start antiviral treatment, although with the combination of pegylated interferon and ribavirin, should be the presence of moderate graft fibrosis (F2 METAVIR or S3 Ishak).[6] The gold standard in the assessment of liver fibrosis progression is to perform protocol liver biopsies at variable time intervals. Although the ideal time interval between liver biopsies is not settled, due to the compressed natural history of recurrent hepatitis C, some authors have advocated yearly intervals.[7]

Liver biopsy is an invasive procedure that has been associated with potential risks of morbidity and in rare cases of mortality; furthermore it is not often easily accepted by the patient. Thus, non-invasive tools for the assessment of liver fibrosis and fibrogenesis are much needed. Among the current adopted methodsto non-invasively assess liver fibrosis

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progression after LT, indirect tests based on the combinations of several biochemical parameters and imaging techniques are more frequently employed.[8] The latter are represented by transient elastography (Fibroscan) and by Acoustic Radiation Force Impulse (ARFI). Both methods have been extensively investigated for the assessment of liver fibrosis in immune competent patients.[9-12] Fibroscan and ARFI have the same accuracy as liver biopsy in identifying patients with cirrhosis on the one hand or patients with minimal or no fibrosis on the other.[13] While Fibroscan has been studied in liver transplanted patients[14,15] the accuracy of ARFI in the assessment of liver fibrosis progression after LT has been investigated in adults in two reports.[16,17]

This study aimed:

- To assess, using liver biopsy as a reference standard, the accuracy of ARFI in discriminating HCV positive liver transplanted patients with different graft fibrosis severity and
- To compare the performance of ARFI with that of several indirect non-invasive biochemical tests in the assessment of liver fibrosis.

MATERIAL AND METHODS

Patients

This prospective study included 51HCV positive liver transplanted patients who consecutively underwent to either per protocol or on demand liver biopsy, to assess the graft status from October 2011 to April 2014 in the Medical Liver Transplant Unit, University of Udine, Italy. The main clinical and demographic characteristics of the studied population are presented in table 1. All patients were maintained in an immunosuppressive regimen that was either cyclosporine- or tacrolimus-based, associated, in the first few months, to corticosteroids. None of the patients considered in the study taken statins or other drugs able to increase the transaminases values and none of the patients at the moment of ARFI measurement or liver biopsy were on antiviral therapy. The study plan was to enroll all these patients for ARFI examinations to be performed concomitantly with liver biopsy and blood chemistry tests. The study has been approved by the local ethics committee and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons gave their informed consent prior to their inclusion in the study.

Table 1. Main demographic and clinical characteristics of studied population (n = 51). Continuous variables are presented as median (interquartile range) and categorical variables as frequencies (%).

Recipient age, years	64.7 (58.8-69.6)
Recipient male gender	40 (78.4%)
Recipient BMI, kg/m^2	25.8 (24.1-28.2)
Recipient abdominal perimeter, cm	100 (95-104)
HCV	
Genotype 1-4	36 (70.6%)
Genotype 2-3	15 (29.4%)
Immunosuppressive therapy	
Tacrolimus	27 (52.9%)
Cyclosporine	24 (47.1%)
AST (IU/L)	30 (23-43)
ALT (IU/L)	27 (20-54)
γGT (IU/L)	55 (29-98)
PLT (x1,000/mm^3)	137 (105-166)
Previous antiviral therapy for HCV recurrence (N.)	30 (58.8%)
Time from liver transplant to last liver biopsy (mo.)	97 (48-146)
Portal tracts	10 (8-14)

BMI: body mass index. HCV: hepatitis C virus. AST: aspartate-aminotransferase. ALT: alanine-aminotransferase. γGT: gamma glutamyl-transpeptidase. PLT: platelets.

ARFI elastography

ARFI was conducted twice by two expert operators (A and B) during real-time B-mode imaging to ensure that other intrahepatic structures, i.e. large vessels, are not included; all patients underwent ARFI measurement in fasting conditions. Siemens Acuson S2000TM ultrasound system (Siemens AG, Germany), software version 2.0 with a 4 CI ultrasound probe was employed. Scanning was performed in the right liver lobe, being patients in supine position, 1-2 cm under the liver capsule, with minimal scanning pressure applied by the operator. All ARFI measurements were obtained at the end of deep inspiration. In all patients 20 valid acquisitions for each site were obtainedand a median value was calculated, the result being expressed in m/s. Reliable ARFI measurements were defined as: median value of 20 valid measurements with a SR ≥ 60% and an IQR < 30%.

Liver biopsy

Ultrasound guided liver biopsy was performed in the same session of ARFI measurements using Biomol 17 G needle in local anesthesia. The liver biopsy specimens were evaluated by a single expert pathologist, blinded to the results of ARFI measurements. Grading and staging were graded by Ishak score.[18]

Laboratory investigations and non-invasive fibrosis tests

Fasting serum levels of alanine (ALT), aspartate (AST) aminotransferase, gamma glutamyl-transpeptidase (γGT)

Table 2. Bonacini test.

Parameters/score	0	1	2	3	4	5	6
INR	< 1.1	1.1 - 1.4	> 1.4				
ALT/AST ratio	> 1.7	1.7 - 1.2	1.19 - 0.6	< 0.6			
PLT x 1000/mm^3	> 340	340 - 280	279 - 220	219 - 160	159 - 100	99 - 40	< 40

and platelet count were measured before performing liver biopsy. Serum biochemical parameters included in the calculation of the following non-invasive test to assess liver fibrosis were also measured:[19]

- **AST/ALT ratio (AAR index) =** AST/ALT.
- **APRI (AST to platelet ratio index) =** [(AST, upper limit of normal)/platelet count (10^9/L)] x 100.
- **Fibrotest =** 4,467 x log[alpha2 – macroglobulin (g/l)] - 1,357 x log[haptoglobin(g/l)] + 0.0821 x [age (years)] + 1737 x log[bilirubin(μmol/l)] – 1,184 x [apolipoprotein A1 (g/l) + 0.301 x sex (male = 1, female = 0) - 5.054.
- **Fibroindex =** 10.929 + (1.827 x Ln AST) + (0.081 x age) + (0.768 x past alcohol use) + (0.385 x HOMA - IR) - (0.447 x cholesterol).
- **Bonacini.** See table 2.
- **Lok index =** -5.56 - 0.0089 x platelet (10^3mm^3) + 1.26 x AST/ALT ratio + 5.27 x INR.
- **Age/platelet score=** age (years) (<30 = 0; 30-39 = 1; 40 - 49 = 2; 50 - 59 = 3; 60 - 69 = 4; 70 = 5)+platelet count (10^9/L) (225 = 0; 200 - 224 = 1; 175 - 199 = 2; 150 - 174 = 3; 125 - 149 = 4; < 125 = 5).
- **FibroQ =** [(10 x age (years) x AST x PT INR) / (PLT x ALT)].
- **HUI model =** 3.148 + 0.167 x BMI + 0.088 x bilirubin - 0.151 x albumin - 0.019 x platelet.
- **GUCI =** normalized ASTxprothrombin - INR x 100/ platelet count (x 10^9/L).

Statistical analysis

Statistical analysis of data was performed using the BMDP dynamic statistical software package 7.0 (Statistical Solutions, Cork, Ireland) and MedCalc version 13.1.2. Continuous variables were presented either as mean (± S.E.) or median (interquartile ranges, IRQ). Categorical variables have been presented as frequencies (%). Differences in frequencies were evaluated by means of χ^2 test. Analysis of variance and Mann-Whitney test were adopted to analyze continuous variables with either a parametric or non-parametric approach. The correlation between Ishak staging scores and ARFI values was assessed by means of the *r* correlation coefficient. ROC curves and the corresponding AUCs were calculated and used to choose the best cut-offs in discriminating different degrees of graft fibrosis. Sensibility, specificity, PPV and NPV and Youden index were therefore calculated. Comparisons for ROC curves were also performed.

RESULTS

Liver histology

Median (IRQ) time interval elapsed from liver transplant and the liver biopsy, performed in the same day of ARFI measurement, was 97 (48-146) months. The median (IRQ) of portal tracts included in liver specimens was10 (8-14). The cumulative median (IRQ) Ishak grading and staging scores was 3 (2-4) and 1 (0-3) respectively. The majority of liver biopsies (38/51) presented a low degree of liver fibrosis (Ishak score 0-2). The cumulative median (IRQ) percentage of graft steatosis was 5% (4-10%). No significant histologic features of acute cellular rejection were recorded in any liver biopsy.

ARFI elastographyand Ishak staging scores

Median (IRQ) of ARFI values was 1.315 m/sec (1.110 - 2.135) for the operator A and 1.530 m/sec (1.195 - 2.290) for the operator B (Spearman rank coefficient 0.828, $p < 0.001$ between the two operators). Median (IRQ) of ARFI values

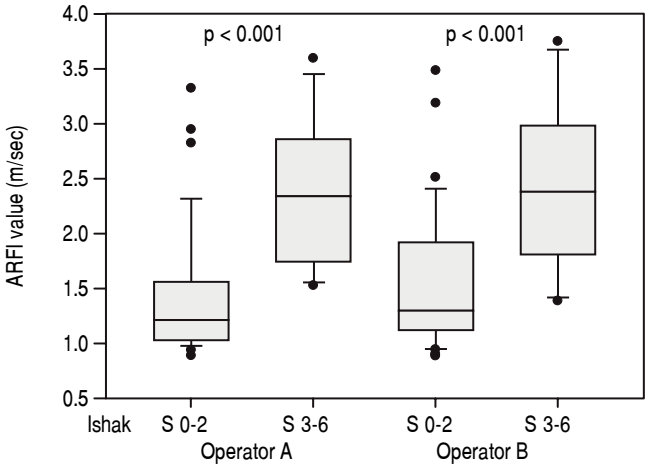

***Figure 1**. Box plots representing the median (IRQ) of ARFI measurements (m/sec) observed in two different categories of Ishak fibrosis scores: 0-2, and 3-6. Data are presented separately for operator A and operator B. The statistical analysis was performed by means of Mann-Whitney test.*

stratified byIshak S 0-2 to 3-6 were 1.215 m/sec (1.031 - 1.558), and 2.335 m/sec (1.733 - 2.853) (Mann-Whitney $p < 0.001$) by operator A and 1.295 m/sec (1.120-1.911) and 2.380 m/sec (1.805 - 2.980) (Mann-Whitney $p<0.001$) by operator B (Figure 1).

A significant correlation was detected between ARFI values obtained by operator A and Ishak staging scores ($r = 0.675$, $p < 0.0001$) (Figure 2).

Non-invasive test of liver fibrosis

Table 3 illustrates the results of 11 non-invasive tests according to Ishak staging scores intervals (S0 - 2 to S3 - 6). In four of the tests employed (Fibro-test, Bonacini score, Lok score and Age/platelet score) a significant difference in the values was detected between patients with low (S0-2) and high (S3-6) fibrosis scores.The largest differences were encountered assessing Fibro-test and Age/platelet score. Therefore these two tests were further evaluated in the paper.

ROC curves were constructed to assess the capacity of non-invasive tests to discriminate between patients with or without significant fibrosis (S0 - 2 *vs.* S3 - 6). The best AUCs was obtained by ARFI (0.885), followed by Fibro-test

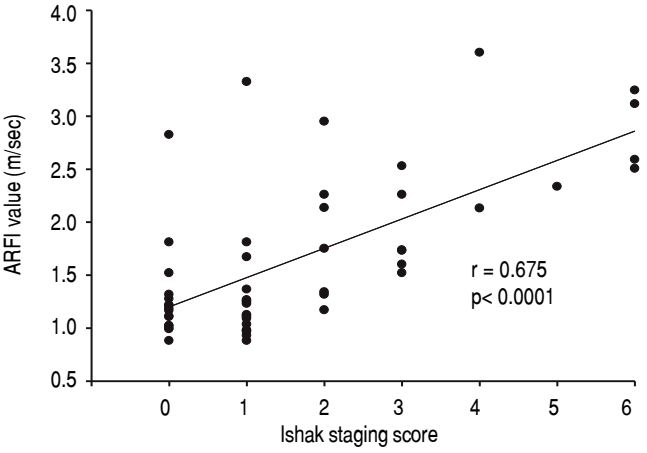

***Figure 2**. Scatterplot between Ishak staging scores and ARFI values (m/sec) obtained by operator A. The regression line is represented as well as the corresponding r value.*

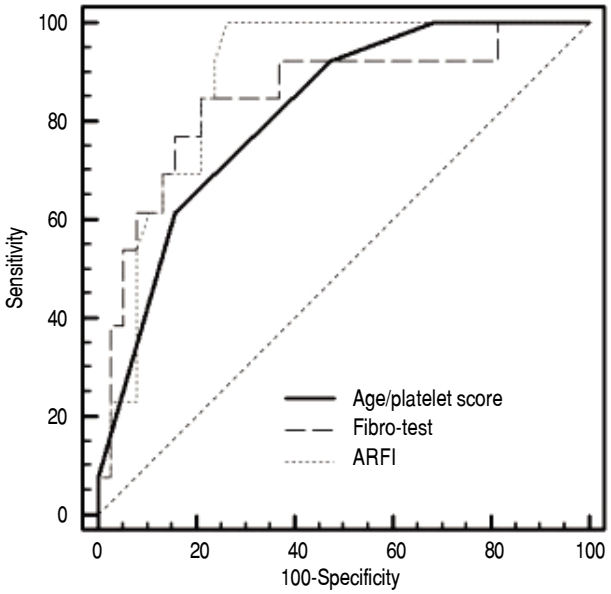

***Figure 3.** Comparison of ROC curves for Age/platelet score, Fibro-test and ARFI in discriminating between patients with graft Ishakfibrosis ≤ 2 and those with Ishak fibrosis ≥ 3. Significance of pairwise comparisons of ROC curves: Fibro-test* vs.*age/platelet score p = 0.720; ARFI vs. age/platelet score p = 0.291. Fibro-test* vs. *ARFI p = 0.611.*

Table 3. Non-invasive test results and ARFI measurement (operator A and B) in relationship to the degree of liver fibrosis (Ishak 0-2 and 3-6). Data are presented as mean (± SE) and evaluated by means of analysis of variance.

	Ishak staging scores		Analysis of variance	
	0-2 (n =38)	3-6 (n =13)	F	p
Non-invasive tests				
AST/ALT ratio	1.17 ± 0.09	1.16 ± 0.11	0.00	0.962
ALT/AST ratio	0.97 ± 0.05	0.98 ± 0.11	0.02	0.886
APRI	0.96 ± 0.27	1.53 ± 0.40	1.27	0.266
Fibro-test	0.50 ± 0.04	0.81 ± 0.05	17.7	< 0.001
Fibro-index	2.39 ± 0.12	2.81 ± 0.21	3.06	0.087
Bonacini score	5.74 ± 0.18	6.62 ± 0.31	6.06	0.017
Lok score	0.48 ± 0.03	0.61 ± 0.05	4.12	0.048
Age/platelet score	7.05 ± 0.25	8.62 ± 0.21	12.5	<0.001
FibroQ	5.92 ± 0.83	8.21 ± 1.22	2.07	0.156
HUI	4.12 ± 0.18	4.78 ± 0.21	3.87	0.055
GUCI	1.02 ± 0.29	1.69 ± 0.47	1.39	0.244
ARFI				
Operator A	1.41 ± 0.10	2.38 ± 0.18	25.0	< 0.001
Operator B	1.55 ± 0.10	2.47 ± 0.21	19.4	<0.001

Table 4. Fibro-test, Fibro-index, Bonacini score, Lok score, Age/platelet score, HUI and ARFI ROC results in discriminating between patients with graft Ishak fibrosis score 0-2 and those with graft Ishak fibrosis score 3-6.

Ishak ≤ 2 / ≥ 3	AUC	SE	p	Cut-off	Sensitivity	Specificity	PPV	NPV	J index
Fibro-test	0.848	0.069	<0.001	0.716	84.6	78.9	57.9	93.7	0.636
Fibro-index	0.686	0.088	0.034	2.950	53.8	81.6	50.0	83.8	0.354
Bonacini score	0.711	0.079	0.008	6	53.8	78.9	46.7	83.3	0.328
Lok score	0.696	0.080	0.014	0.483	76.9	63.2	41.7	88.9	0.401
Age/platelet score	0.816	0.059	<0.001	8	61.5	84.2	57.1	86.5	0.457
HUI	0.696	0.077	0.011	3.577	100	36.8	35.1	100	0.368
ARFI	0.885	0.046	<0.001	1.365	100	73.7	56.5	100	0.737

(0.848) and Age/platelet score (0.816) (Figure 3). Using a cut-off of 1.365 m/sec, ARFI possesses sensitivity and NPV of 100% in discriminating patients with significant fibrosis (≥ S3).Using a cut-off value of 0.716, Fibro-test possesses sensitivity and NPV of 84.6% and 93.7% respectively in discriminating patients with significant liver fibrosis (Table 4). The combined assessment of ARFI and Fibro-test did not improve the results obtained by ARFI alone.

DISCUSSION

To evaluate tissue stiffness, the Virtual Touch tissue quantification system, which uses ARFI technology, has been introduced and is now commercially available in an ultrasound apparatus.[20] The system uses a standard real-time B mode ultrasonography probe and offers elastography with a flexible metering box of 1 cm at variable depths in the liver parenchima. This box is defined the region of interest (ROI). This region is graphically displayed at a size of 10 mm long by 6 mm wide and can be placed at the discretion of the operator both in the left and in the right lobe of the liver. Generally the operator positioned the probe over the ROI in the segment VIII of the right lobe of the liver, approximately 2 cm from the liver capsule away from motion and portal/hepatic vessels. Tissue in the ROI is mechanically excited with an impulsive acoustic radiation force, which results in the generation of shear waves within the tissue; the velocity of these shear waves, which is proportional to the square root of the tissue elasticity, is quantitatively expressed as the tissue stiffness in m/s. A great advantage of ARFI is that the software is included in an ultrasound device, therefore a simultaneous ultrasound and Doppler ultrasound of the liver can be performed with the same probe. Ultrasound guidance is also particularly helpful for ensuring that the ROI is placed in such a way that it avoids nearby vessels and ribs. This represents a significant advantage especially when the region of interest measured by ARFI is smaller than it is for Fibroscan. Fibroscan failed to obtain effective results in about 11% of patients and this is primary due to the presence of obesity and/or ascites.[16] These conditions seem to be less relevant in influencing ARFI results.[9] In fact, in our series all patients had a valid ARFI measurement independently from their body mass index. A further facility of ARFI measurement is that the results obtained are promptly available and therefore can be communicated to the patients.

Several reports have been published dealing with the usefulness of ARFI in the assessment of liver fibrosis in patients with HCV related chronic hepatitis.[9,21] In these patients ARFI has been found to be an accurate tool, effective as Fibroscan, in discriminating patients with significant liver fibrosis or cirrhosis from those with minimal or mild fibrosis.[13] Our results highlighted the accuracy of ARFI for measuring the degree of liver fibrosis in the set of LT. The ARFI AUROC in discrimination significant liver fibrosis was 0.885. ARFI was found to possess sensitivity and negative predictive value of 100% in discriminating patients with Ishakliver fibrosis ≤ 2 / ≥ 3 (cut-off value of 1.365 m/sec).The results obtained in our study are comparable to those previously reported by Crespo, *et al*.[16] who observed an AUROC of 0.902 in discriminating patients with Scheuer F ≤ 1 / ≥ 2. Moreover, our data emphasized thatARFI is operator independent thus being considered fairly reproducible.

Several non-invasive markers of liver fibrosis have been proposed, individually or in an algorithm-based score, instead of liver biopsy. These markers are usable in clinic, because the physicians are able to request the analyses of them during the treatment and for assessing the treatment efficacy, too.[8] In the set of LT, they have the potential to become an important tool in monitoring liver fibrosis progression in HCV positive recipients, thus reducing the need for per-protocol liver biopsies.[8] Although in HCV positive liver transplanted patients specific non-invasive tests have been constructed and validated, they performed similarly to those commonly adopted in predicting liver fibrosis stage in HCV positive immune-competent.[22] Among the latter, in our previous experience, APRIvalue > 1.4 was 91% sensitive and 75% specific in detecting Ishak graft fibrosis score > 2 in HCV positive liver transplanted patients.[23]

In the present study, Fibrotest with AUROC of 0.848 was found to be the best performer among non-invasive tests in discriminating patients with significant fibrosis. Fibrotest has been extensively investigated in HCV positive immune-competent, in whom it gave clinically relevant results.[24] Only in two studies, enrolling HCV positive liver transplanted patients, Fibrotest has been evaluated as a non-invasive tool to predict liver fibrosis stage.[14,25] Compared to Fibrotest results obtained in these two studies, our data highlighted a better performance of the test. Although the difference in the accuracy of Fibrotest between our study and that conducted by Beckembaum, *et al*.[25] was found to be relatively low, Corradi, *et al.*[14] obtained very poor results, probably because of the small number of patients involved.

One might argument against the distribution in the degree of liver fibrosis in our cohort. In fact the majority of the patients enrolled presented mild or no fibrosis at liver biopsy; on the contrary cirrhosis was seldom recorded. Although this criticism could appear formally correct, several considerationsshould be applied to limit this drawback. The diagnosis of graft cirrhosis in clinical practice can be easily made without the need of liver biopsy; for this reason the number of cirrhotic patients who underwent histologic examination of the liver was relatively low. Indeed, a same low proportion of cirrhoticswas invariably present in the studies that dealt on this topic of investigation.[14,16]

Liver biopsy is considered the gold standard in the assessment of liver fibrosis. However it has been demonstrated that the accuracy of liver biopsy in the evaluation of liver fibrosis is influenced by the site of the biopsy, by the number of portal tracts present in the sample and by the experienceof the pathologist in the evaluation.[26] This reflects a large variability in the AUROC of liver biopsy in discriminating patients with significant liver fibrosis among studies conducted on this topic. At this regard it must be underlined that in our series the median number of portal tracts present in the biopsy specimens was 10, a value as near as optimal, and that an experienced pathologist evaluated all the biopsies.

On the clinical point of view the major issue of interest arising in the decision making processing of these patients is toidentify those patients without significant liver fibrosis in whom a watchful waiting approach may be applied and an expensive antiviral therapy could be delayed without the risk of graft loss.This goal could be obtainable without the need of protocol liver biopsies in a substantial number of recipients, applying only ARFI measurements. Adding to ARFI other non-invasive liver fibrosis tests such as Fibrotest does not appear to improve the accuracy in identifying patients in whom antiviral therapy could be delayed.

CONCLUSION

ARFI measurement in HCV positive liver transplanted patients can be considered an easy and accurate non-invasive tool in identifying patients with a benign course of HCV recurrence.

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