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ORIGINAL ARTICLE

Hepatitis C Virus Reinfection in Liver Transplant Patients: Evaluation of Liver Damage Progression with Echo-Color Doppler

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Liver transplant recipients are a model of rapid progression of hepatitis C virus (HCV)–related liver disease, from normal to cirrhosis. The aim of the study was the analysis of the relationship between portohepatic hemodynamics and modification in liver histology during the progression of HCV liver disease after transplant. Patients transplanted for HCV cirrhosis were considered for the study. At least every 6-12 months, the portal blood flow velocity, hepatic and splenic pulsatility indices, and a portal hypertensive index (obtained from the combination of the portal blood velocity and splenic pulsatility index) were measured with echo-Doppler. Liver biopsy was performed whenever necessary. The time course of echo-Doppler parameters during the histological progression of the liver disease was analyzed. Posttransplant patients without HCV were included as controls. Forty-nine patients with histology-proven relapse of HCV hepatitis were included in the study. At the onset of recurrent hepatitis, the portal blood flow velocity significantly decreased ($P < 0.001$), and the splenic pulsatility index increased ($P = 0.020$), whereas the hepatic pulsatility index remained unchanged. In the following years, in addition to a further slight decrease in the portal blood velocity ($P = 0.027$), a progressive increase in the hepatic and splenic pulsatility indices was also detected ($P = 0.009$ and $P < 0.0001$, respectively). The portal hypertensive index steadily increased with the progression of the disease and was related to the degree of liver fibrosis. In conclusion, the information obtainable from splanchnic Doppler parameters can be used to monitor the progression of liver fibrosis in transplant patients with HCV reinfection. ***Liver Transpl 14:616-624, 2008.*** © **2008 AASLD.**

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Splanchnic hemodynamics of liver transplant patients with relapse of hepatitis C virus (HCV) chronic hepatitis or cirrhosis have not been investigated. Knowledge of these data may be useful in diagnosing the recurrence of HCV hepatitis and in monitoring the progression of the disease.

The progression of liver damage after HCV reinfection in patients transplanted for HCV cirrhosis is faster than in infected nontransplant patients. In nontransplant patients, HCV infection spontaneously recovers in 15% of the patients, causes an asymptomatic disease with normal aminotransferases in about 25% of the patients, and progresses to chronic hepatitis in 60% of cases; the progression from chronic hepatitis to cirrhosis is then quite slow, occurring on average in 20% of patients after 10-20 years.[1,2] On the contrary, in patients transplanted for HCV cirrhosis, HCV reinfection of the graft is almost constant.[3] The relapse of chronic hepatitis is common, occurring on average in 75%-80% of the patients after 5 years, and the progression of liver damage is faster, with a 10%-30% prevalence of cirrhosis 5 years after transplant.[4,5]

This study was aimed at analyzing the changes in splanchnic hemodynamics provoked by the recurrence

Abbreviations: ANOVA, analysis of variance; DUSCI, Doppler ultrasound composite index; F, fibrosis stage; HCV, hepatitis C virus; NS, not significant; OLT, orthotopic liver transplantation; PBF, portal blood flow volume; PBV, portal blood flow velocity; PHI, portal hypertensive index; PI, pulsatility index.

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and progression of HCV disease in patients with orthotopic liver transplantation (OLT). The relationship between hemodynamic parameters and liver histology was also analyzed.

PATIENTS AND METHODS

Patients and Design of the Study

All patients 18 years old or older who underwent OLT for HCV cirrhosis and who were referred to our department between January 1993 and October 2005 were considered for the study. Because in the first weeks after transplant splanchnic hemodynamic parameters undergo important modifications regardless of the presence of complications,[6] patients were considered eligible for the study only 1 month after the intervention. This was considered the minimum time necessary to obtain a stabilization of splanchnic hemodynamic parameters. Moreover, considering patients only 1 month after OLT allowed the exclusion of the very first period after transplant, when other complications, such as acute rejection, are more frequently responsible for hepatic dysfunction than a relapse of HCV hepatitis.[7]

Patients were excluded if they were affected by vascular alterations, such as portal or arterial thrombosis or stenosis, which are known to modify per se splanchnic Doppler parameters.[8,9]

Patients underwent echo-Doppler evaluation according to a predetermined schedule: every 1-3 months during the first year after OLT and every 12 months thereafter over the following years. Duplex Doppler examination was also performed every time the clinical situation necessitated it.

A protocol liver biopsy was performed in all patients 6 months after OLT. Liver biopsy was also performed whenever there was clinical and biochemical deterioration suggestive of liver dysfunction: increased aminotransferase, bilirubin, alkaline phosphatase, and γ-glutamyltransferase levels. Patients who had a protocol biopsy 6 months after OLT but did not show recurrent hepatitis were included at a later time point if they had histologic HCV recurrence. Only patients with a histologically proven relapse of HCV hepatitis were included in the study. They were included at the first sign of hepatitis at histology and were then followed up in the subsequent years. Histology of subsequent biopsies was recorded, and a possible evolution to cirrhosis was documented.

The choice of including patients only 1 month after transplantation and the reliance on clinically indicated biopsies probably caused an underestimation in the actual rate of recurrence and progression of the disease. Nevertheless, the detection of recurrence and progression rates was not the aim of the study, and we wanted to decrease any confounding factor.

No biopsy with histologic signs consistent with clinically relevant rejection, corresponding to a rejection activity index of 4 or greater,[10] was included.

Posttransplant patients with non–virus-related liver disease and without complications after surgery were included as controls.

To identify the splanchnic Doppler parameters related to the degree of liver fibrosis and the grade of inflammation, which are potentially useful in transplanted patients, we preliminarily performed a cross-sectional study, comparing splanchnic Doppler parameters and liver histology in 80 nontransplant patients with biopsy-proven HCV chronic liver disease without clinical sign of overt cirrhosis (male/female: 49/31; age: 46 ± 11 years).

Then, the relationships between Doppler parameters and liver histology were analyzed in transplant patients with relapse of HCV liver disease.

The time course of echo-Doppler parameters during the histological progression of the liver disease was also analyzed in transplant patients with HCV disease in whom more than 1 biopsy was performed at intervals of 1 to 3 years after the relapse of HCV hepatitis.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. No donor organs were obtained from executed prisoners or other institutionalized persons.

Normal values of Doppler parameters were obtained from a group of 45 healthy subjects (male/female: 18/17; age: 45 ± 13 years) who did not show alteration in liver function tests, a history of liver disease, or alteration in liver, spleen, or portal morphology.

Measurement of Echo-Doppler Parameters

Splanchnic hemodynamic parameters were evaluated with echo-color Doppler. A Toshiba Sonolayer SSA-270A (Toshiba, Tokyo, Japan) with color Doppler sonography and a 3.75-MHz sector electronic probe was initially used, and an HDI 5000 (ATL, Seattle, Washington) with color Doppler sonography and a broadband curved array transducer (C5-2 40R) was used after October 1998.

Parameters were measured in suspended normal respiration. Portal blood flow velocity (PBV) was evaluated as the time-averaged maximum velocity multiplied by the coefficient 0.57, assuming the portal velocity profile to be parabolic, as previously reported.[11,12] Portal blood flow volume (PBF) was obtained by multiplication of the portal vein cross-sectional area, assuming a circular shape of the portal vein section, by PBV.[11,12] The arterial Doppler resistance indices were measured as pulsatility index (PI) = (peak systolic velocity − end diastolic velocity)/mean velocity.[13] Hepatic artery PI values were measured in the left and right intrahepatic branches of the hepatic artery and reported as the average between the values in the 2 branches.[14] Splenic PI values were measured near the hilum of the spleen, as previously reported.[12] Spleen size was estimated by the bipolar diameter.[15]

A new index, calculated as splenic PI divided by PBV, was measured in every patient and was defined as the portal hypertensive index (PHI). In the evaluation of the progression of liver fibrosis, the Doppler ultrasound composite index (DUSCI) was also calculated by sub-

traction of the percentage change in splenic PI from the percentage change in PBV.[16]

The ranges of normal values of splanchnic echo-Doppler parameters, obtained from 45 healthy subjects (age: 45 ± 13 years), were as follows: PBV, 15-35 cm/s; PBF, 300-1200 mL/minute; hepatic PI, 0.64-1.20; splenic PI, 0.54-0.92; PHI, 0.025-0.055 s/cm; and spleen size, 8-12 cm.

Interobserver and intraobserver variability of splanchnic echo-Doppler measurements in our center has already been reported.[12,14,17]

Histological Study

All liver biopsies were obtained with a modified Menghini aspiration biopsy set and a 16-gauge needle. All specimens were formalin-fixed, paraffin-embedded, and routinely stained with hematoxylin-eosin, periodic acid-Schiff after diastase digestion, and Van Gieson stain for collagen. All biopsy specimens of both transplant and nontransplant subjects were re-examined by the same experienced pathologist, who was unaware of the clinical status of the subjects or of Doppler results. Criteria for the diagnosis of recurrent HCV hepatitis included the presence of HCV-RNA in serum associated with consistent histological findings, which included lobular necroinflammatory lesions and/or mononuclear portal/periportal inflammation.[18] The stage of fibrosis and grade of inflammation were assessed according to Ishak et al.[19] For the aim of this study, fibrosis scores of 5 (that is, fibrous septa with severe architectural disturbance–incomplete cirrhosis) and 6 (that is, cirrhosis) were considered a single group. The differential diagnosis from other possible complications was based on accepted criteria.[18] Because liver biopsies were collected retrospectively, all samples were considered, regardless of their size. Portal tracts were counted in all cases, and none of the included biopsy samples contained less than 5 complete portal tracts.

Statistical Analysis

Results are reported as mean ± standard deviation. Differences among groups were assessed by 1-way analysis of variance (ANOVA) and, if significant, with the Newman-Keuls test. A test for the linear trend between the mean and column number was also performed to evaluate the relationships between the echo-Doppler parameters and Ishak score for liver fibrosis. Correlations were investigated by nonparametric correlation (Spearman). A progression in liver fibrosis was defined as an increase of at least 2 points in the Ishak score between 2 subsequent liver biopsies. When we compared data from 2 groups of patients, the Student t test for paired or unpaired samples was used. The observed distribution of numbers was compared with the hypothesized distribution by the chi-square test. Sensitivity, specificity, and positive and negative predictive values were calculated according to the usual formulas. The null hypothesis was rejected if P values < 0.05 were found.

RESULTS

Among the patients who underwent an OLT in the study period, 84 were affected by HCV-related cirrhosis and were considered eligible for the study. Thirty-five patients were excluded from the analysis. Five patients were excluded because of vascular alteration, that is, arterial or portal thrombosis or stenosis. Twenty-one patients did not undergo the 6-month protocol biopsy because of the following: 4 patients had already died; 9 patients were referred to another liver transplant center; and 8 patients had a clinically significant biopsy-proven acute rejection[10] in the weeks preceding the scheduled protocol biopsy, which was not performed. Among the remaining 9 patients in whom the 6-month protocol biopsy did not show recurrence of HCV hepatitis, 1 was subsequently lost to follow-up (he was referred to another liver transplant center), 6 did not show subsequent significant alanine aminotransferase flare, 1 had a clinically significant acute rejection, and 1 had a cholangitis. No case of severe cholestatic hepatitis C was detected among these patients.

Forty-nine patients were included in the final analysis (male/female: 40/9; age: 52 ± 10 years). Two patients had transjugular intrahepatic portosystemic shunt before transplant. During the intervention, no one needed superior mesenteric vein or splenic vein anastomosis or venous grafts. All these patients had histology-proven relapse of HCV hepatitis (average time after OLT: 497 ± 591 days; median time: 251 days). One patient had biopsy-proven relapse of HCV cirrhosis 1273 days after OLT, without a prior biopsy demonstrating a relapse in chronic hepatitis. Twelve of the 48 patients with relapse of HCV chronic hepatitis evolved into cirrhosis (average time after OLT: 1289 ± 872 days; median time: 1348 days; average time after the relapse of chronic hepatitis: 868 ± 641 days; median time: 479 days).

The 12 patients with recurrence of HCV hepatitis that later evolved into cirrhosis had higher values of aminotransferases with respect to the other 38 patients at the onset of hepatitis recurrence: aspartate aminotransferase (normal values 10-45 U/L), 203 ± 232 versus 55 ± 42 ($P = 0.0018$), and alanine aminotransferase (normal values 10-50 U/L), 290 ± 317 versus 106 ± 88 ($P = 0.006$).

Sometimes, it was not possible to measure all Doppler parameters because of the low quality of echo-Doppler visualization (insufficient visualization makes splanchnic Doppler measurements not reliable). Therefore, in the 100 biopsies performed in the 49 patients and included in the final analysis, PBV and PBF were not available 6 times, hepatic PI was not available 2 times, splenic PI was not available 4 times, and PHI was not available 9 times. In 7 biopsies (out of 100), evidence of recurrent HCV disease coexisted with signs of mild allograft rejection (rejection activity index ≤ 3), which needed no specific antirejection therapy.

As a control group, we selected 28 non-HCV transplant patients without complications after surgery (male/female: 19/9; age: 49 ± 10 years; etiology: 19

TABLE 1. Comparison of Echo-Doppler Hemodynamic Parameters in the Investigated Patients with HCV Hepatitis

	Non-HCV Transplant Patients (n = 28)	Transplant Patients with HCV Recurrent Hepatitis (n = 48)	*P*
Portal blood flow mean velocity (cm/s)	20.4 ± 5.7	15.8 ± 5.1	0.0006
Portal blood flow volume (mL/minute)	1317 ± 303	1155 ± 473	NS
Hepatic pulsatility index	1.20 ± 0.34	1.21 ± 0.39	NS
Splenic pulsatility index	0.80 ± 0.15	0.88 ± 0.19	0.069
Portal hypertension index (s/cm)	0.043 ± 0.013	0.062 ± 0.025	0.0001
Spleen size (cm)	13.1 ± 2.7	14.4 ± 2.4	0.036

NOTE: In the group of HCV transplant patients, the reported parameters are those collected at the diagnosis of recurrent HCV hepatitis. In the group of non-HCV transplant patients, the reported parameters are those collected 1 year after surgery. In the HCV group, 1 transplant patient with cirrhosis was not included in this table.
Abbreviations: HCV, hepatitis C virus; NS, not significant.

alcoholic cirrhosis, 3 primary sclerosing cholangitis, 2 primary biliary cirrhosis, and 4 other nonviral etiologies). Transplant patients without HCV were analyzed 1 year after the intervention, a time span similar to that of transplant patients with HCV recurrence (average time after OLT for biopsy-proven recurrence of HCV hepatitis: 497 ± 591 days; median time: 251 days).

Comparison Between Splanchnic Hemodynamic Parameters in HCV Transplant Patients with Recurrence of HCV Hepatitis and Non-HCV Transplant Patients

With respect to the 28 non-HCV transplant patients, transplant patients with HCV recurrent hepatitis had a hemodynamic pattern characterized by lower PBV, similar PBF, and hepatic PI (Table 1); splenic PI was slightly but not significantly increased, whereas PHI was markedly increased (Table 1).

Time Course of Splanchnic Doppler Parameters in Patients with Recurrent HCV Hepatitis

At the onset of recurrent hepatitis, PBV significantly decreased (from 22.4 ± 8.2 to 15.8 ± 5.1 cm/s, $P < 0.001$), splenic PI significantly increased (from 0.79 ± 0.19 to 0.88 ± 0.19, $P = 0.020$), and hepatic PI showed a slight and not significant (NS) increase (from 1.16 ± 0.41 to 1.21 ± 0.39, P = NS). PHI increased from 0.044 ± 0.023 to 0.062 ± 0.025 s/cm ($P < 0.001$). Twenty-seven patients were followed up for up to 3 years after the relapse of HCV chronic hepatitis, 23 were followed up for up to 4 years, and 21 were followed up for up to 5 years. In the years following the relapse of HCV chronic hepatitis, a progressive further decrease in PBV was detected in the first 3 years of follow-up (one-way ANOVA: $P = 0.027$; posttest for linear trend: $P = 0.0034$) and not subsequently (Fig. 1). A progressive increase in hepatic PI values was detected only after an analysis of the 5-year follow-up (one-way ANOVA: $P = 0.009$; posttest for linear trend: $P = 0.008$; Fig. 1). On the contrary, a progressive increase in splenic PI values was detected after an analysis of the 3-year follow-up (one-way ANOVA: $P = 0.02$; posttest for linear trend: $P = 0.04$) and the 4-year follow-up and 5-year follow-up (one-way ANOVA: $P < 0.0001$ and $P < 0.0001$; posttest for linear trend: $P < 0.0001$ and $P < 0.0001$, respectively; Fig. 1). PBF remained unchanged. PHI further and steadily increased in the years following the diagnosis of relapse of HCV hepatitis (3-year follow-up: one-way ANOVA: $P = 0.0014$; posttest for linear trend: $P = 0.0002$; 4-year follow-up: one-way ANOVA: $P = 0.0002$; posttest for linear trend: $P < 0.0001$; 5-year follow-up: one-way ANOVA: $P < 0.0001$; posttest for linear trend: $P < 0.0001$; Fig. 1).

Analyzing the 12 patients in whom chronic hepatitis evolved into cirrhosis, we compared Doppler values collected at the last examination performed before the diagnosis of cirrhosis, when severe chronic hepatitis was already present, with values collected at the time of diagnosis of cirrhosis. At the onset of cirrhosis, PBV changed from 13.7 ± 5.8 to 11.7 ± 3.9, $P = 0.052$; hepatic PI values changed from 1.18 ± 0.40 to 1.32 ± 0.49, P = NS; splenic PI values changed from 0.92 ± 0.17 to 1.05 ± 0.25, P = NS; and PHI changed from 0.077 ± 0.030 to 0.098 ± 0.030 s/cm, $P = 0.001$.

Splanchnic Doppler parameters in patients with relapse of HCV chronic disease after OLT were not significantly influenced by the immunosuppressive regimen or by possible hypotensive or antiviral therapy. In particular, among the 49 HCV transplant patients included in the study, 15 underwent antiviral therapy (interferon/pegylated interferon–ribavirin) during the follow-up. Among these 15, only 3 achieved a sustained virological response. Changes over time of Doppler parameters were not statistically different in these 3 groups. PHI values at the recurrence of HCV hepatitis and then after 1, 2, 3, 4, and 5 years were as follows (s/cm): for 34 HCV patients without antiviral therapy, 0.063 ± 0.015, 0.065 ± 0.015, 0.065 ± 0.022, 0.080 ± 0.040, 0.092 ± 0.033, 0.103 ± 0.034, and 0.081 ± 0.018; for 12 HCV patients with antiviral therapy but without sustained virological response, 0.084 ± 0.033,

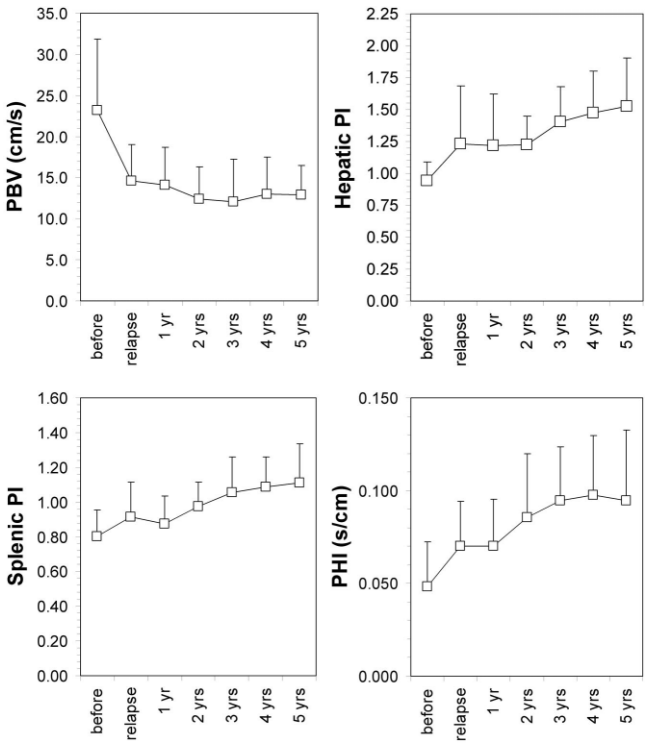

Figure 1. Time course of splanchnic Doppler parameters in transplant patients after the recurrence of HCV hepatitis. Twenty-seven patients were followed up for up to 3 years, 24 were followed up for up to 4 years, and 21 were followed up for up to 5 years. In the years following the relapse of HCV chronic hepatitis, a progressive further decrease in PBV was detected in the first 3 years of follow-up (one-way ANOVA: $P = 0.027$; posttest for linear trend: $P = 0.0034$) and not subsequently. A progressive increase in hepatic PI values was detected only with an analysis of the 5-year follow-up (one-way ANOVA: $P = 0.009$; posttest for linear trend: $P = 0.008$). On the contrary, a progressive increase in splenic PI values was detected with an analysis of the 3-year follow-up (one-way ANOVA: $P = 0.02$; posttest for linear trend: $P = 0.04$) and the 4-year follow-up and 5-year follow-up (one-way ANOVA: $P < 0.0001$ and $P < 0.0001$; posttest for linear trend: $P < 0.0001$ and $P < 0.0001$, respectively). PHI further and steadily increased in the years following the diagnosis of relapse of HCV chronic hepatitis (3-year follow-up: one-way ANOVA: $P = 0.0014$; posttest for linear trend: $P = 0.0002$; 4-year follow-up: one-way ANOVA: $P = 0.0002$; posttest for linear trend: $P < 0.0001$; 5-year follow-up: one-way ANOVA: $P < 0.0001$; posttest for linear trend: $P < 0.0001$). Abbreviations: ANOVA, analysis of variance; HCV, hepatitis C virus; PBV, portal blood flow velocity; PHI, portal hypertensive index; PI, pulsatility index.

0.080 ± 0.029, 0.099 ± 0.026, 0.098 ± 0.028, 0.097 ± 0.031, and 0.123 ± 0.053; and for 3 HCV patients with antiviral therapy with sustained virological response: 0.065 ± 0.026, 0.066 ± 0.033, 0.069 ± 0.015, 0.099 ± 0.015, 0.072 ± 0.008, and 0.075 ± 0.015.

Relationship Between Echo-Doppler Parameters and Liver Fibrosis and Inflammation

In the preliminary cross-sectional group of 80 nontransplant patients with HCV chronic liver disease, liver fibrosis was related to PBV, splenic PI, and PHI but not to hepatic PI (Table 2 and Fig. 2). Liver inflammation was not related to PBV (ANOVA: $F = 0.726$, $P = 0.69$) or hepatic PI (ANOVA: $F = 0.8396$, $P = 0.59$), whereas there was a correlation with splenic PI (ANOVA: $F = 3.648$, $P = 0.0006$), and there was a weak correlation with PHI (ANOVA: $F = 2.164$ $P = 0.030$).

The relationship of liver fibrosis with PBV and PHI was confirmed in transplant patients with HCV chronic disease (Table 3 and Fig. 2). The relationship was also maintained without the biopsies with coexistence of mild rejection being taken into account (fibrosis score and PBV: ANOVA: $F = 4.931$, $P = 0.0013$; test for linear trend: $r = 0.38$, $P = 0.0003$; Spearman correlation: $r = -0.43$, $P < 0.0001$; fibrosis score and PHI: ANOVA: $F = 7.732$, $P < 0.0001$; test for linear trend: $r = 0.49$, $P < 0.0001$; Spearman correlation: $r = 0.50$, $P < 0.0001$). On the contrary, no correlation was found in HCV transplant patients between Doppler parameters and liver inflammation (PBV: ANOVA: $F = 0.755$, $P =$ NS; hepatic PI: ANOVA: $F = 1.560$, $P =$ NS; splenic PI: ANOVA: $F = 1.508$, $P =$ NS; PHI: ANOVA: $F = 1.59$, $P =$ NS). Therefore, in transplanted patients with HCV recurrence, PHI is related to the score of fibrosis but not to the grade of inflammation.

High and low values of PHI allowed the identification of patients with high and low degrees of fibrosis, respectively (chi-square test: $P = 0.0004$ in nontransplant patients, $P < 0.0001$ in transplant patients; Fig. 3).

Considering an absolute PHI value > 0.104 s/cm as the cutoff for predicting fibrosis stage 5 (F5)/F6 in the graft, we obtained a sensitivity of 50%, a specificity of 94%, a positive predictive value of 58%, and a negative predictive value of 91%. With a lower cutoff value (PHI > 0.083), the sensitivity increased, but the positive predictive value further decreased: a sensitivity of 71%, a specificity of 73%, a positive predictive value of 32%, and a negative predictive value of 93%. Therefore, the use of an absolute PHI value has only a high negative predictive value for predicting F5/F6 in the graft.

Relationship Between Changes in the Echo-Doppler Parameters and Progression of Liver Fibrosis

Thirty-five of the patients with relapse of HCV hepatitis after transplant underwent at least another liver biopsy after 1 to 3 years. When a progression of liver fibrosis was detected between 2 subsequent biopsies (a worsening of at least 2 points in the Ishak score; n = 17), PHI increased more than when there was no progression of fibrosis (n = 24; +70% ± 67% versus +20% ± 38%, $P = 0.004$). DUSCI was also higher when there was a progression of fibrosis (44% ± 45% versus 14% ± 26%, $P = 0.009$; Fig. 4).

The degree of progression in fibrosis, evaluated as an increase in the Ishak score, was related to the change in PHI (Spearman r: 0.45, $P = 0.003$) and to the value of DUSCI (Spearman r: 0.46, $P = 0.0022$).

In this group of patients, an increase in PHI of at least 50% identified the patients with a progression of liver

TABLE 2. Relationship Between Doppler Parameters and the Degree of Liver Fibrosis Evaluated with the Ishak Score in the 80 Nontransplant Patients

	F1 (n = 10)	F2 (n = 27)	F3 (n = 26)	F4 (n = 9)	F5-F6 (n = 8)	One-Way ANOVA	Test for Linear Trend	Nonparametric Correlation (Spearman)
PBV (cm/s)	18.9 ± 3.0	18.0 ± 5.3	15.5 ± 3.0	13.1 ± 2.3†	14.9 ± 3.4	$F = 4.38$	$r = 0.33$	$r = -0.46$
						$P = 0.002$	$P = 0.002$	$P < 0.0001$
Hepatic PI	1.06 ± 0.24	1.04 ± 0.16	1.14 ± 0.18	1.14 ± 0.15	1.08 ± 0.15	$F = 1.32$	$r = 0.07$	$r = 0.19$
						$P = 0.27$	$P = 0.51$	$P = 0.086$
Splenic PI	0.74 ± 0.08	0.81 ± 0.10	0.86 ± 0.17*	0.94 ± 0.08†	0.95 ± 0.17†	$F = 5.14$	$r = 0.42$	$r = 0.48$
						$P = 0.001$	$P < 0.0001$	$P < 0.0001$
PHI (s/cm)	0.040 ± 0.008	0.048 ± 0.013	0.059 ± 0.016†	0.075 ± 0.022‡	0.066 ± 0.018†	$F = 9.15$	$r = 0.48$	$r = 0.58$
						$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Abbreviations: ANOVA, analysis of variance; F, fibrosis stage; PBV, portal blood flow mean velocity; PHI, portal hypertensive index; PI, pulsatility index.
*$P < 0.05$ in comparison to F1 (according to the Newman-Keuls multiple comparison test).
†$P < 0.05$ in comparison to F1 and F2 (according to the Newman-Keuls multiple comparison test).
‡$P < 0.05$ in comparison to F1, F2, and F3 (according to the Newman-Keuls multiple comparison test).

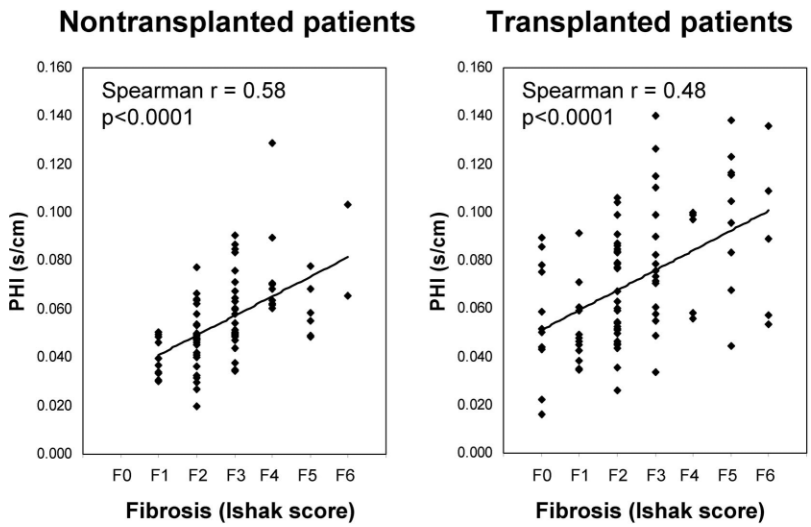

Figure 2. Relationship between PHI and the stage of liver fibrosis in 80 biopsies performed in 80 nontransplant patients with HCV chronic hepatitis and in 91 biopsies performed in 49 transplant patients with relapse of HCV liver disease. Abbreviations: F, fibrosis stage; HCV, hepatitis C virus; PHI, portal hypertensive index.

fibrosis with a sensitivity of 71% (12/17), a specificity of 88% (21/24), a positive predictive value of 80% (12/15), and a negative predictive value of 81% (21/26).

DISCUSSION

This study shows that at the onset of HCV chronic hepatitis in transplant patients, a decrease in PBV and an increase in splenic PI can be detected. Hepatic PI values increase with the subsequent evolution of the disease. Through a combination of Doppler parameters, it is possible to obtain an index that seems capable of monitoring not only the onset of the relapse of HCV hepatitis but also the subsequent progression of liver fibrosis. Therefore, splanchnic echo-Doppler may be a useful diagnostic tool in the evaluation of the progression of HCV chronic hepatitis after OLT.

In this study, splanchnic hemodynamics have been investigated in the same patients from the healthy stage to the evolution of HCV chronic hepatitis and then cirrhosis. This kind of analysis is not feasible in nontransplant patients because of the long evolution time of HCV infection (about 20-40 years).[1,2] Therefore, transplant patients offer the unique possibility of studying splanchnic hemodynamic evolution from normal to cirrhosis in the same patient. On the other hand, we have to keep in mind that the hemodynamic patterns of transplant and nontransplant patients are different. Indeed, also in the absence of complications, patients transplanted for liver cirrhosis show a persistent increase in portal inflow, probably due to the persistence of splenomegaly, with an increase in hepatic artery resistance indices, which may be linked to the arterial buffer response to increased PBF.[6]

After HCV relapse, splanchnic Doppler parameters showed a progressive worsening over time, possibly reflecting a progression of the disease.

In this study, we also verified the relationships between Doppler parameters and liver fibrosis and the relationships between the modification in Doppler parameters and the progression of liver fibrosis.

TABLE 3. Relationship Between Doppler Parameters and the Degree of Liver Fibrosis Evaluated with the Ishak Score in the 100 Biopsies Performed in the 49 Transplant Patients

	F0 (n = 15)	F1 (n = 12)	F2 (n = 36)	F3-F4 (n = 22)*	F5-F6 (n = 15)	One-Way ANOVA	Test for Linear Trend	Nonparametric Correlation (Spearman)
PBV (cm/s)	16.3 ± 4.5	18.3 ± 5.3	15.4 ± 4.8	12.9 ± 4.4‡	11.6 ± 3.7‡	$F = 4.78$	$r = 0.35$	$r = -0.41$
						$P = 0.015$	$P = 0.0004$	$P < 0.0001$
Hepatic PI	1.20 ± 0.47	1.09 ± 0.30	1.28 ± 0.39	1.20 ± 0.31	1.30 ± 0.45	$F = 0.76$	$r = 0.10$	$r = 0.11$
						$P = 0.55$	$P = 0.32$	$P = 0.30$
Splenic PI	0.85 ± 0.27	0.89 ± 0.16	0.94 ± 0.16	0.96 ± 0.16	1.02 ± 0.24	$F = 1.82$	$r = 0.27$	$r = 0.19$
						$P = 0.13$	$P = 0.009$	$P = 0.06$
PHI (s/cm)	0.056 ± 0.024	0.052 ± 0.016	0.066 ± 0.021	0.082 ± 0.027†	0.095 ± 0.031†	$F = 8.04$	$r = 0.47$	$r = 0.48$
						$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Abbreviations: ANOVA, analysis of variance; F, fibrosis stage; PBV, portal blood flow mean velocity; PHI, portal hypertensive index; PI, pulsatility index.
*Transplant patients with F3 and F4 fibrosis were analyzed together because only 5 patients had F4 fibrosis.
†$P < 0.05$ in comparison to F0, F1, and F2 (according to the Newman-Keuls multiple comparison test).
‡$P < 0.05$ in comparison to F1 (according to the Newman-Keuls multiple comparison test).

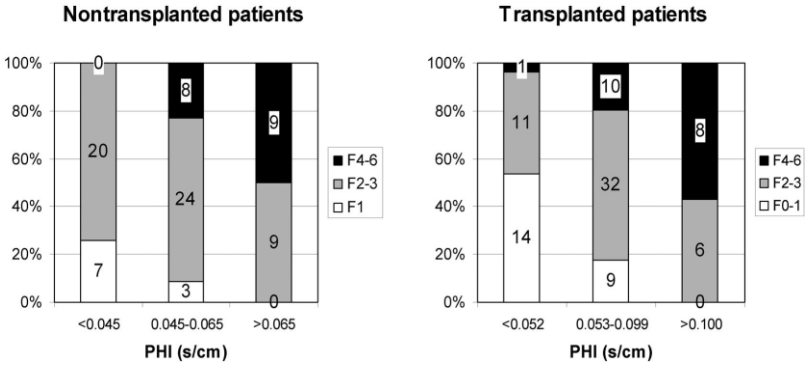

Figure 3. Distribution of the degree of liver fibrosis among patients divided according to the level of PHI in transplant and in nontransplant patients. High and low values of PHI allowed the identification of patients with high and low degrees of fibrosis, respectively (chi-square test: $P = 0.0004$ in nontransplant patients, $P < 0.0001$ in transplant patients). Abbreviations: F, fibrosis stage; PHI, portal hypertensive index.

Published studies that have focused on the relationship between Doppler ultrasonography and the degree of liver fibrosis in chronic hepatitis have given conflicting results, but they are not easily comparable because different parameters and different clinical settings have been evaluated. Decreased PBV has been shown to be related to significant fibrosis by some authors[20-25] but not by others.[26-28] The hepatic resistance index, which is higher in cirrhosis than in chronic hepatitis,[29,30] is influenced by the extent of fibrous tissue deposition in the liver according to some authors[23,28,31] but not to others.[26] Hepatic vein spectrum was related to liver fibrosis in 2 studies[24,25] and was not related to it in another 3 studies.[23,26,32] It is possible that the lack of homogeneity in the study results may be accounted for, at least in part, by the variability of Doppler measurements. This problem can be overcome by specific training for Doppler operators.[11,33] Few studies have evaluated the clinical usefulness of splenic PI in the monitoring of chronic hepatitis. Among patients with chronic hepatitis, Piscaglia et al.[34] demonstrated that the splenic resistance index can identify those patients with a relevant portal hypertension (presence of gastroesophageal varices). Liu et al.[28] demonstrated that the splenic resistance index and PI show significant changes with the advance of hepatic fibrosis and that they are useful for the detection of significant hepatic fibrosis in HCV carriers with persistently normal alanine aminotransferase. A few authors have tried to analyze the usefulness of composite indices, that is, those combining more than 1 Doppler parameter. The index combining PBV with the hepatic resistance index was related to the severity of liver fibrosis,[25] whereas the index combining arterial and portal blood velocity gave conflicting results.[26,35] Piscaglia et al.[34,36] proposed an index combining hepatic and splenic PI values with PBV. The index was accurate in predicting the progression from chronic hepatitis to cirrhosis with esophageal varices.[34]

We could find no data in the literature about the relationships between splanchnic Doppler parameters and liver fibrosis in transplant patients. In this study, we propose the use of a simple obtainable index, the PHI, which combines the 2 Doppler parameters that are modified with the change in portal resistance, that is, PBV and splenic PI.

In the evaluation of the progression of liver fibrosis, DUSCI was also calculated.[16] DUSCI can be considered an unspecific tool to evaluate the increase in portal resistance, and it has already proved its usefulness in the evaluation of the onset of acute rejection after liver transplant.[16]

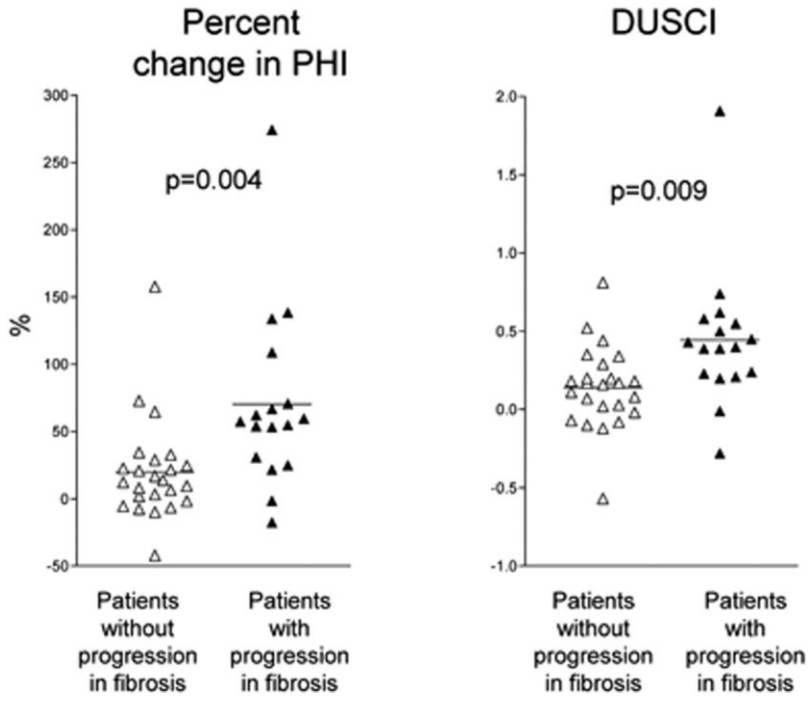

Figure 4. Evaluation of the variation in PHI (calculated as splenic PI divided by PBV) and DUSCI (calculated by subtraction of the percentage change in splenic PI from the percentage change in PBV) in transplant patients with or without progression of liver fibrosis after the relapse of hepatitis C virus hepatitis. Abbreviations: DUSCI, Doppler ultrasound composite index; PBV, portal blood flow velocity; PHI, portal hypertensive index; PI, pulsatility index.

In the cross-sectional study of nontransplant patients, we found a significant positive trend when comparing the degree of fibrosis with splanchnic Doppler parameters and with PHI in particular. Despite the high variability of Doppler parameters in transplant patients, PHI maintained a significant relationship with the degree of fibrosis, probably because its calculation compensates the variability of the single measurements.

The most interesting result of our study is the relationship between the increase in PHI and the progression in liver fibrosis after the relapse of HCV hepatitis in transplant patients. The worse the progression of fibrosis was, the higher the increase in PHI was. These findings underline the importance of evaluating changes over time of Doppler parameters more than single absolute values. This is particularly important in a condition such as that of transplant patients because splanchnic hemodynamics may differ very much from one patient to another.

After the first months after OLT, splanchnic Doppler parameters tend to stabilize if no complication occurs.[6] Therefore, if an increase in PHI is detected in a patient transplanted for HCV cirrhosis, a progression in liver fibrosis should be suspected, and further investigations should be prompted.

Even though the investigated group is small and the result certainly has to be validated in other groups of patients, possibly in other centers, it seems that this parameter may be able to identify, among patients with HCV reinfection, those with a progression of fibrosis.

To have simply obtainable parameters able to identify patients with a progression of fibrosis before the onset of cirrhosis would be of paramount importance for the obvious therapeutic consequences. We cannot give data about possible sudden marked increases in PHI in patients with early ascites and more severe hepatitis C recurrence because we did not observe early ascites in the included patients. The onset of ascites was observed only after the relapse of cirrhosis.

The usefulness of a prescheduled protocol of Doppler ultrasound measurement in transplant patients is once more underlined, as it could allow the identification not only of patients with vascular complications[8,9] but also of patients with suspected parenchymal complications, such as acute liver rejection[16] and relapse of HCV chronic disease. If our data are confirmed, PHI could represent an additional noninvasive test useful in the identification of patients with relapse of HCV chronic hepatitis and of patients with a progression of the degree of fibrosis.

In conclusion, the information obtainable from splanchnic Doppler parameters can be combined in a single index that increases with the progression of liver fibrosis in transplant patients with HCV reinfection.

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