The Hepatitis Aggressiveness Score (HAS)

A Novel Classification System for Post–Liver Transplantation Recurrent Hepatitis C

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Abstract: Several histopathologic features have been described in cases of fibrosing cholestatic hepatitis C (FCH-C). We investigated whether FCH-associated features can be utilized as the basis of a novel grading system for the entire population of post–liver transplantation (LT) recurrent hepatitis C virus (HCV) infection. Liver biopsies obtained at a median (interquartile range) of 12.3 (10.4-13.8) months post-LT from 170 patients with recurrent HCV were included. Biopsies were assessed for the following FCH features: (1) ductular reaction, (2) cholestasis, (3) hepatocyte ballooning, and (4) periportal sinusoidal fibrosis. A Hepatitis Aggressiveness Score (HAS) was assigned on the basis of the number of FCH features as follows: 0 features = HAS 1; 1 to 2 features = HAS 2; and 3 to 4 features = HAS 3. We analyzed the performance of this novel system in predicting clinicopathologic outcomes compared with conventional grading systems after a median (interquartile range) follow-up of 24 (13-45.5) months. The HAS classification was highly predictive of fibrosis progression ($P < 0.001$) and was the best predictor of graft loss in a multivariable analysis model, which included all conventional hepatitis grading systems (adjusted hazard ratio = 5.5, confidence interval 2.9-10.7, $P < 0.001$ for HAS 3 vs. HAS 1 and 2, compared with adjusted hazard ratio = 1.0, confidence interval 0.5-1.9, $P = 0.94$ for the presence of moderate to severe necroinflammation by at least 1 conventional grading system). Presence of at least 3 of 4 FCH features (HAS 3 group) characterized a subset of patients with distinctly worse prognosis and severe cholestatic disease (ie, FCH-C). We propose a novel approach to the histologic grading of post-LT recurrent HCV based exclusively on FCH features. This system allows accurate identification of FCH-C cases and stratification of all recurrent HCV patients into distinct prognostic categories.

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Chronic hepatitis C virus (HCV) infection is currently the most common indication for liver transplantation (LT) in the United States, representing the underlying liver disease in over one third of allograft recipients.[1] Post-LT recurrent hepatitis C is nearly universal and represents an important complication in this population.[2–7] Allograft HCV infection, however, is in many respects biologically distinct from HCV infection of native livers, largely because of an altered immune response to HCV in the setting of post-LT immunosuppression. Well-described differences include various aspects of viral kinetics, significantly faster rate of fibrosis progression, and more frequent development of highly aggressive, cholestatic variants of the disease, referred to as fibrosing cholestatic hepatitis C (FCH-C).[8–12] Histologically, the severity of HCV-related necroinflammation is assessed according to the same grading systems used to evaluate chronic hepatitis in native livers and represents one of several parameters used to monitor post-LT disease activity, shown to correlate with subsequent progression to advanced fibrosis and cirrhosis in some studies.[3,13–15] Histologic grade, however, has inconsistently been incorporated into clinical algorithms for initiation of antiviral treatment in the post-LT setting.[12,16–18] Moreover, histologic features associated with the most severe forms of recurrent HCV infection (ie, FCH-C)[10,19–23] are not included in conventional grading systems and, therefore, when present, must be evaluated separately.

Although various morphologic features of FCH-C are well described in literature, the precise number and severity of findings needed for the histopathologic characterization of this condition have not been established. The 2003 International Transplant Society (ITS) expert panel consensus conference on LT and hepatitis C recommended the utilization of histopathologic features as

part of their suggested clinicopathologic diagnostic criteria of FCH-C, including "ballooning of perivenular hepatocytes, paucity of inflammation, and variable degree of cholangiolar proliferation without bile duct loss."[12] These criteria, however, may lack sufficient detail to be useful in the evaluation of individual cases by pathologists, and their diagnostic utility, alone or in combination, has not been well established. As a result, studies to date have inconsistently reported various combinations of histopathologic findings of FCH-C, if any, in conjunction with clinical parameters.[22] Therefore, more specific recommendations regarding the histopathologic characterization of FCH-C have become essential.

In this study, we have reviewed liver biopsies from a historical cohort of 170 patients diagnosed with recurrent HCV infection to investigate whether histopathologic evaluation of FCH-associated features can be used as the basis of a prognostically meaningful classification system for the entire population of recurrent hepatitis C patients and whether a more precise histopathologic characterization of FCH-C on the basis of clinicopathologic correlation can be established.

PATIENTS AND METHODS

Patient Selection

Medical records of all patients who underwent LT for HCV-related end-stage liver disease at our institution from February 2002 to December 2009 were assessed. All liver biopsies from HCV-infected patients performed between 6 and 18 months post-LT and all samples reported as "consistent with FCH" or "showing features of FCH" at any time after LT were rereviewed. If >1 sample was obtained during this period, the sample with the highest degree of necroinflammatory activity was selected as the "index biopsy." Post-LT HCV infection was defined by a positive HCV RNA by polymerase chain reaction (PCR). A positive HCV PCR in only the pre-LT period was also considered acceptable in the absence of HCV antiviral therapy.

Exclusion criteria were the following: (a) evidence of therapy-related HCV clearance before biopsy; (b) significant biliary complication (defined as abnormal biliary imaging by endoscopic retrograde cholangiopancreatography or magnetic resonance cholangiopancreatography requiring endoscopic, percutaneous, or surgical intervention); (c) evidence of acute cellular rejection as the primary histopathologic abnormality (for exclusion of any given sample as "index biopsy"); (d) evidence of concomitant disease(s) that could influence the natural history of post-LT recurrent hepatitis C, including viral hepatitis B coinfection, human immunodeficiency virus coinfection, steatohepatitis (defined by the presence of at least 2 of the following features in addition to steatosis: predominantly centrilobular hepatocyte ballooning, predominantly centrilobular sinusoidal fibrosis, and Mallory hyaline), and chronic ductopenic rejection (defined by the presence of interlobular bile ducts in $<50\%$ of portal tracts in a sample containing ≥ 10 portal tracts) (e) death or retransplantation within 30 days of LT; and (f) unavailable or inadequate (<5 portal tracts present in the sample) liver biopsy material.

Clinical Data

All clinical, laboratory, and follow-up data utilized in this study were obtained from our institution's electronic medical records. Serum quantitative HCV RNA levels (Cobas Amplicor HCV v2.0; Roche Diagnostics, Branchburg, NJ) were recorded if measured within 1 month of the index biopsy. All other laboratory data used in this study correspond to prebiopsy levels, collected within 1 week of the liver biopsy date. The term "graft loss" refers to either patient death or graft failure requiring liver retransplantation. Graft survival time is measured in months from the index biopsy to either graft loss or last follow-up. This study was approved by our center's institutional review board.

Histopathologic Data

Archival formalin-fixed paraffin-embedded 4-μm-thick tissue sections of liver biopsy samples from each patient were evaluated by routine (hematoxylin and eosin) stains and special stains (including Masson trichrome and reticulin stains). Biopsies were independently graded and staged according to the Batts-Ludwig,[24] Ishak [modified Hepatitis Activity Index, (HAI)],[25] Scheuer,[26] and Metavir[27] systems by 2 pathologists (R.K.M. and M.S.), who were blinded to clinical and outcome data. Discrepant scores were resolved by consensus rereview of pertinent slides. In addition, all biopsies were also assessed for the presence of the following histopathologic features, which comprised the basis of our proposed grading system:

1. Prominent ductular reaction, at least focally expanding portal tracts, mimicking biliary obstruction.
2. Prominent hepatocyte ballooning/swelling (with cellular enlargement and cytoplasmic rarefaction), present in the majority of the sample, with lobular disarray.
3. Cholestasis (including at least focal canalicular cholestasis), of any degree.
4. Periportal sinusoidal/pericellular fibrosis (excluding areas of "usual" pattern of periportal fibrosis).

Each of the features was described as present or absent in each biopsy (as illustrated in Fig. 1). The final Hepatitis Aggressiveness Score (HAS) was assigned as follows: HAS 1—presence of 0 criterion; HAS 2—presence of 1 to 2 criteria; HAS 3—presence of 3 to 4 criteria (Table 1). For both ductular reaction and hepatocyte ballooning, the qualifier "prominent" was adopted in this study, as mild cases of either feature may be difficult to recognize (and may require cytokeratin immunohistochemistry in the case of mild to moderate ductular reaction). In addition, fibrosis staging information was collected for all patients who underwent follow-up biopsies (ie, after index biopsy) from our pathology database. When multiple follow-up biopsies were performed, the highest documented fibrosis stage was recorded.

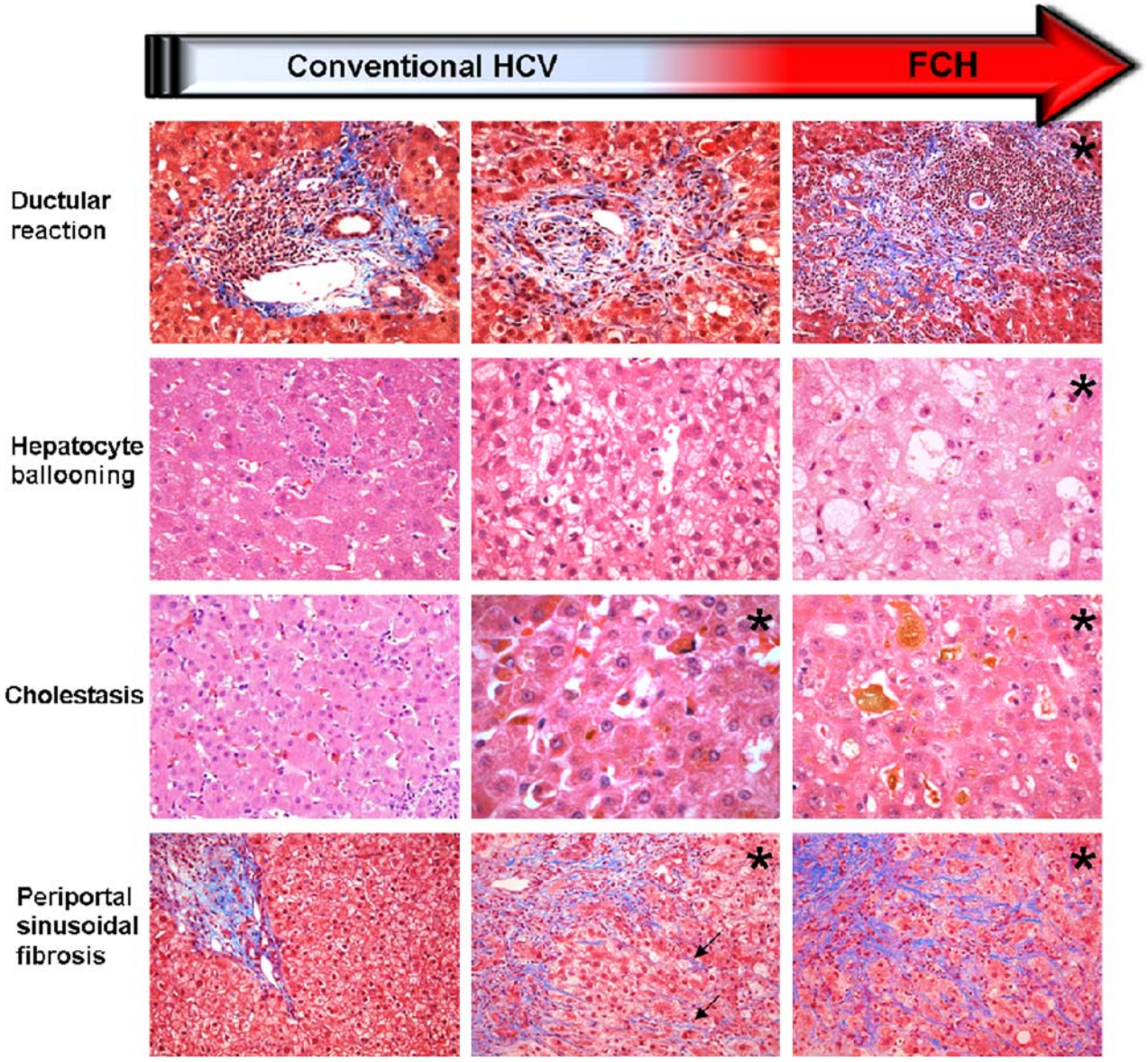

FIGURE 1. Spectrum of FCH-C histopathologic changes in post-LT recurrent hepatitis C ranging from conventional histopathologic appearance (left column) to well-developed FCH-C (right column). Rows 1 through 4 illustrate increasing degrees of severity of ductular reaction, hepatocyte ballooning and lobular disarray, cholestasis, and periportal sinusoidal fibrosis (from left to right: none, moderate, and severe; rows 1 and 4, trichrome stain; rows 2 and 3 hematoxylin and eosin stain). Arrows indicate areas of periportal sinusoidal fibrosis (in contrast to areas of "usual" pattern of periportal fibrosis also shown in the image). *Degree of severity of each feature meeting the study criteria.

Statistical Analysis

Continuous variables were described using median and interquartile range (IQR) and compared using the Mann-Whitney test or Kruskal-Wallis test. Categorical variables were compared using the χ^2 test. Time-dependent graft loss was assessed using Kaplan-Meier survival analysis and log rank test using the following 3 categories of necroinflammatory activity: minimal activity, including Batts-Ludwig grades 0 to 1, Scheuer grade 0 to 1, Ishak scores 0 to 4, and Metavir A0; mild activity, including Batts-Ludwig grade 2, Scheuer grade 2, HAI score 5 to 8, and Metavir A1; and moderate to severe activity, including Batts-Ludwig grades 3 to 4, Scheuer grades 3 to 4, HAI score >8, and Metavir A2 to 3. Cox proportional hazards regression was used for multivariable analysis, which included the HAS, Batts-Ludwig, Scheuer, Metavir, and HAI/Ishak classifications, using the optimal cutoff score for predicting graft loss in each grading/staging system. All variables with a P value <0.20 in univariable analysis were initially included in the multivariable model, and nonsignificant variables were removed in a stepwise manner. All P values were

TABLE 1. The HAS

Histologic features
1. Prominent ductular reaction, at least focally expanding portal tracts, mimicking biliary obstruction
2. Prominent hepatocyte ballooning/swelling, present in the majority of the sample, with lobular disarray
3. Cholestasis (including at least focal canalicular cholestasis), of any degree
4. Periportal sinusoidal fibrosis
Final scoring
0 of 4 features: HAS 1 (nonaggressive hepatitis)
1-2 of 4 features: HAS 2 (aggressive hepatitis)
3-4 of 4 features: HAS 3 (FCH)

FCH indicates fibrosing cholestatic hepatitis; HAS, Hepatitis Aggressiveness Score.

2-tailed, and P values <0.05 were considered statistically significant. Statistical analyses were performed using MedCalc for Windows, version 12.0.0.0 (Mariakerke, Belgium) and Stata 12 (College Station, TX).

RESULTS

Clinical Data

A total of 379 HCV-infected patients underwent LT in our institution during the study period. Exclusion criteria and number of patients excluded were the following: <1 month of follow-up after LT (n = 15), presence of biliary complications (n = 57), diagnosis of chronic rejection (n = 5), no liver biopsy performed from 6 to 18 months after LT (n = 145), or no biopsy slides available for review (n = 41). Several patients were excluded on the basis of >1 criterion. A total of 170 patients met the study criteria [male = 125, female = 45, median (IQR) age at transplantation 56.1 (52.2-60.5) y, median (IQR) post-LT time at index biopsy of 12.3 (10.4-13.8) mo, and median (IQR) follow-up time after index biopsy of 24 (13-45.5) mo] and were included in the analysis. Samples initially reported as "consistent with FCH" or "showing features of FCH" included 8 biopsies obtained before 6 months post-LT (2 to 4.8 mo) and 2 biopsies obtained after 18 months post-LT (31.5 and 33 mo). Demographic information of our study population is summarized in Table 2.

Prognostic Value of FCH Features

At least 1 FCH feature was present in 42.4% (72/170) of biopsies (Fig. 2). All individual histologic features evaluated as part of the HAS system were associated with increased risk of graft loss on univariable analysis. Cholestasis was the most ominous finding [hazard ratio (HR) 8.59; 95% confidence interval, (CI) 2.68-27.46; $P < 0.001$], followed by hepatocyte ballooning with lobular disarray (HR 7.27; 95% CI, 3.71-14.23; $P < 0.0001$), ductular reaction (HR 5.29; 95% CI, 2.30-12.17; $P < 0.0001$), and periportal sinusoidal fibrosis (HR 2.75; 95% CI, 1.50-5.04; $P = 0.0001$) (Fig. 3).

Similarly, the total number of FCH features seen on each liver biopsy had a robust correlation with graft loss (Fig. 3). A statistically significant difference was observed between patients showing 1 versus 0 feature (HR = 2.4; 95% CI, 1.0-6.7; $P = 0.03$) and 3 versus 1 features (HR = 4.7; 95% CI, 1.6-13.8; $P = 0.0001$), whereas the survival difference between patients showing 2 versus 1 (HR = 2.1; 95% CI, 0.75-6.01; $P = 0.11$) and 4 versus 3 (HR = 2.3; 95% CI, 0.91-5.90; $P = 0.5$) features did not reach statistical significance.

TABLE 2. Characteristics of Study Population (n = 170)*

Sex (Male/Female)	125/45
Age at transplantation (y)	56.1 (52.2-60.5)
Donor age (y)	50 (39.2-59)
Calculated MELD score	20 (14-27)
Cold ischemia time (min)	480 (324-596)
Acute rejection Banff ≥ 5 (%)	11.2
Total bilirubin (mg/dL)	1.1 (0.8-2.98)
Direct bilirubin (mg/dL)	0.4 (0.2-1.48)
Aspartate aminotransferase (U/L)	64.5 (37.3-159.5)
Alanine aminotransferase (U/L)	60 (11-122)
Alkaline phosphatase (U/L)	158.5 (106.25-268)
γ-Glutamyl transpeptidase (U/L)†	158 (54-398)
HCV viral load (%)‡	
0-7.6 million (IU/dL) (%)	80.8
>7.6 million (IU/dL) (%)	19.1

*Median (IQR), unless otherwise specified.
†γ-Glutamyl transpeptidase levels available in 113 cases.
‡RNA levels available in 120 cases.
y indicates years; MELD, model for end-stage liver disease; min, minutes; HCV, hepatitis C virus.

Fibrosis Progression

Follow-up biopsies were performed in 119/170 patients (HAS 1 to 2, 108/143; HAS 3, 11/27), after a median (IQR) follow-up time of 23.6 (13.3-45) months after index biopsy. The HAS system was able to accurately predict risk of fibrosis progression, which was significantly different among HAS categories (Fig. 4), including any fibrosis progression (1 stage or more), at least 2-stage progression, and progression to cirrhosis. Subgroup analysis of fibrosis progression of patients with no fibrosis or mild fibrosis (defined as portal or periportal fibrosis without bridging) on index biopsy revealed similar results (Table 3).

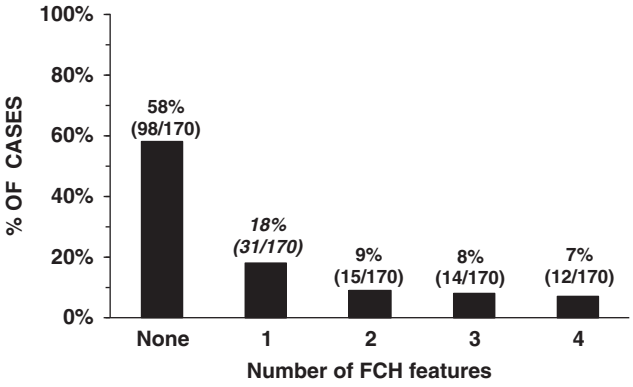

FIGURE 2. Distribution of cases of recurrent hepatitis C showing 0 to 4 FCH histologic features on index biopsy.

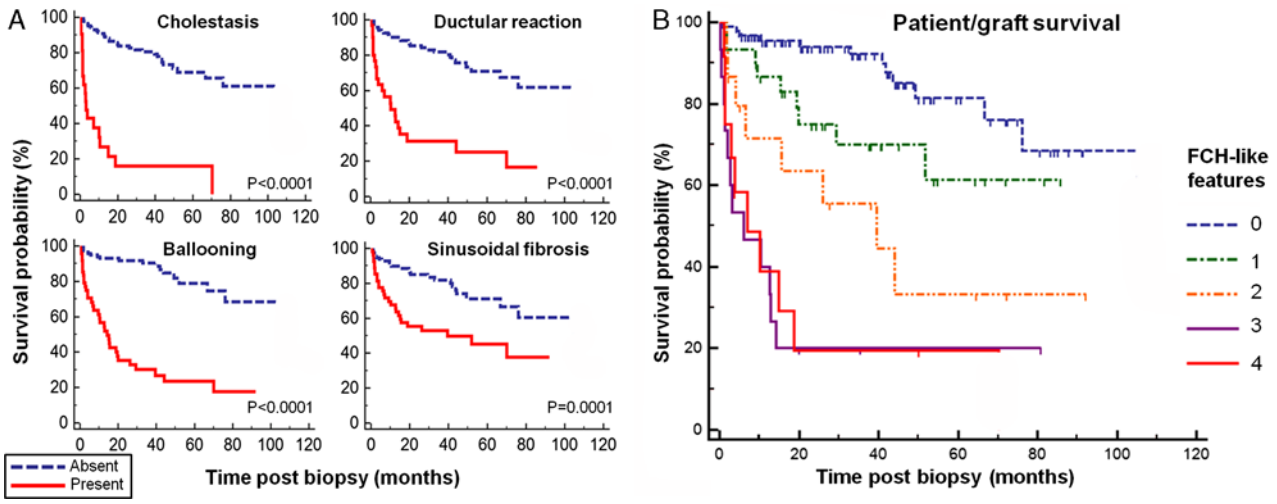

FIGURE 3. A, Survival analysis according to the presence of individual FCH histologic features showing a negative impact of each feature on graft survival. B, Total number of FCH features (ranging from 0 to 4) present on index biopsy accurately predicts graft loss. Statistically different survivals are seen in patients showing 0 versus 1 feature ($P = 0.03$) and 1 versus 3 features ($P = 0.0001$), whereas survival difference between patients showing 1 versus 2 and 3 versus 4 features was not significant. Kaplan-Meier survival analysis and log rank test.

The Hepatitis Aggressiveness Score

Survival analysis allowed us to classify the population of recurrent hepatitis C patients into 3 main prognostic categories. Patients showing none of the analyzed FCH features represented the largest group (98/170; 57.6%) and had a distinctly more favorable prognosis, with 1-year and 3-year graft survival of 95% and 93%, respectively, and protracted course of fibrosis progression, broadly corresponding to the clinically recognized group of "slow fibrosers." This group was classified as HAS 1 or non-aggressive hepatitis. On the opposite end of the severity spectrum were patients with 3 to 4 FCH features (27/170; 15.9%), who showed most of the expected clinicopathologic characteristic of FCH-C, including presence of severe cholestatic disease, and experienced very high and early graft loss, with 1-year and 3-year graft survival of 40% and 20%, respectively. This group was classified as HAS 3 or FCH. Patients showing 1 to 2 features (45/170; 26.4% combined) had an intermediate prognosis compared with the previous groups, with 1-year and 3-year graft survival of 82% and 65%, respectively, and generally corresponded to the clinically recognized group of "non-FCH rapid fibrosers." These patients were grouped into HAS 2 or aggressive hepatitis (Figs. 2, 3).

The 3 HAS groups had significantly different risks of graft loss (HAS 2 vs. 1: HR = 3.2; 95% CI, 1.5-7.0; $P = 0.0007$. HAS 3 vs. 2: HR = 3.9; 95% CI, 1.9-7.9; $P < 0.0001$. HAS 3 vs. 1 to 2: HR = 7.8; 95% CI, 4.4-13.7; $P < 0.0001$). In addition, a 3-tiered classification of recurrent hepatitis C facilitated comparison between the HAS system and existing grading systems, which have typically been divided into 3 groups for statistical

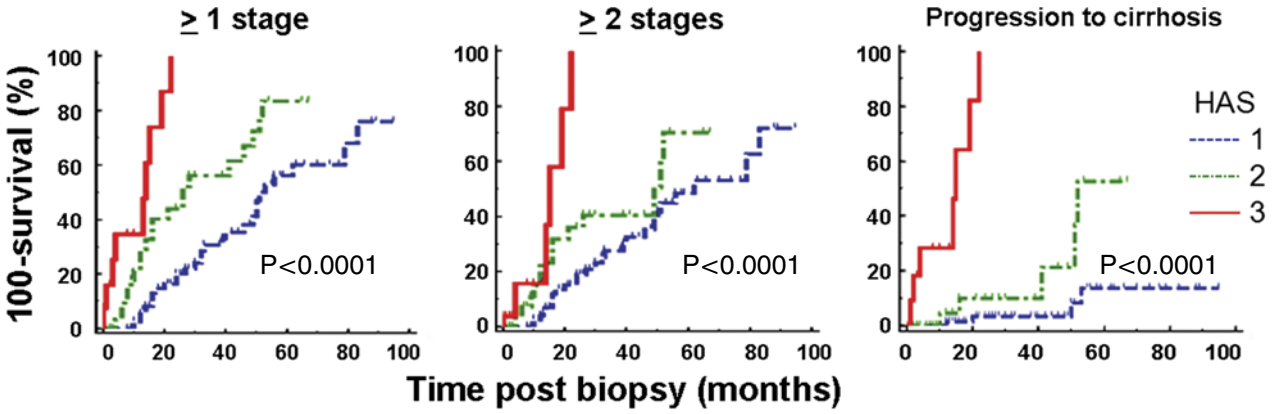

FIGURE 4. Fibrosis progression according to HAS classification (Kaplan-Meier analysis and log rank test). Follow-up biopsies available for 119/170 patients.

TABLE 3. Fibrosis Progression Analysis

	HR	95% CI	*P*
All patients with f/u bx (n = 119)			
Progression of at least 1 stage*			
HAS 2 vs. 1	2.4	1.23-4.74	0.001
HAS 3 vs. 2	2.8	0.91- 8.54	0.007
HAS 3 vs. 1	8.8	1.3-57.2	< 0.0001
Progression of at least 2 stages*			
HAS 2 vs. 1	1.8	0.8-4.0	0.06
HAS 3 vs. 2	2.7	0.67-11.33	0.03
HAS 3 vs. 1	7.7	0.8-74.7	< 0.0001
Progression to cirrhosis*			
HAS 2 vs. 1	4.6	0.9-22.6	0.01
HAS 3 vs. 2	9.5	1.8-51.7	< 0.0001
HAS 3 vs. 1	31.1	2.2-431.5	< 0.0001
Patients with stage ≤2 on index bx (n = 106)			
Progression of at least 1 stage*			
HAS 2 vs. 1	2.8	1.4-5.7	0.0002
HAS 3 vs. 2	2.0	0.6-6.2	0.11
HAS 3 vs. 1	7.5	1-58.6	< 0.0001
Progression of at least 2 stages*			
HAS 2 vs. 1	2.2	1-4.9	0.01
HAS 3 vs. 2	2.4	0.6-9.1	0.07
HAS 3 vs. 1	7.3	0.8-64.8	< 0.0001
Progression to cirrhosis*			
HAS 2 vs. 1	5.4	0.8-35.3	0.01
HAS 3 vs. 2	8.3	1.2-57.7	0.0001
HAS 3 vs. 1	35.1	1.3-984.3	< 0.0001

*Stage ≤2 refers to 4-tiered staging systems and corresponds to no fibrosis, portal fibrosis, or periportal fibrosis without obvious fibrous bridges.
Bx indicates biopsy; f/u, follow-up.

analyses (ie, minimal, mild, and moderate-severe activity) because of the small percentage of cases showing severe necroinflammation in this population.

Correlation With Clinical and Laboratory Data

The median age (IQR) did not differ significantly among the groups [HAS 1: 56.8 (53-61) y; HAS 2: 55.2 (51.1-60.4) y, HAS 3: 55.6 (51.4-59.6) y; $P = 0.78$]. No significant difference in sex distribution, ethnicity, MELD score, and cold ischemia time was noted among the 3 groups (data not shown). The different groups of the HAS classification were associated with increasing median (IQR) levels of total bilirubin [HAS 1: 1.0 (0.7-1.3) mg/dL; HAS 2: 1.2 (0.7-3.5) mg/dL; HAS 3: 6.6 (2.7-17.5) mg/dL; $P < 0.0001$] and direct bilirubin [HAS 1: 0.3 (0.2-0.4) mg/dL; HAS 2: 0.4 (0.2-1.8) mg/dL; HAS 3: 3.3 (1.2-8.5) mg/dL; $P < 0.0001$). Aspartate aminotransferase mean levels were also significantly higher in HAS 3 compared with HAS 1 [135 (99-242) U/L vs. 46.0 (21-77.5) U/L; $P < 0.0001$], whereas no significant difference was found for alanine aminotransferase [HAS 1: 53 (34-84.5) U/L; HAS 2: 67 (40.8-171.5) U/L; HAS 3: 102 (65.5-146.5) U/L; $P = 0.17$], and alkaline phosphatase [HAS 1: 129 (91.5-226) U/L; HAS 2: 181.5 (132.5-277.5) U/L; HAS 3: 256 (138-368.5) U/L; $P = 0.11$]. These results are summarized in Figure 5. Serum HCV RNA levels were available in 61.2% (104/170) of cases. At our institution, however, levels higher than 7.6 million IU/dL are not quantified (reported as >7.6 million IU/dL instead). For this reason, HCV RNA levels were subdivided into 2 categories (> or <7.6 million IU/dL). Among non-FCH patients (HAS 1 to 2), 10.5% of patients (9/86) had HCV RNA levels above this level, compared with 44.4% (8/18) of FCH-C (HAS 3) patients ($P = 0.0002$). Within the FCH-C/HAS 3 group, patients with HCV RNA levels >7.6 million IU/dL showed a trend toward higher rates of graft loss compared with patients with lower viral loads [HR = 2.61 (0.8-6.7); $P = 0.08$]. Serum cytomegalovirus (CMV) PCR was performed in 24/27 HAS 3 cases within a median of 3.5 (1-6) weeks of the index biopsy and was negative in all cases. Immunohistochemical studies for CMV were not performed in any of the HAS 3 cases (no CMV cytopathic effect was identified on hematoxylin and eosin-stained slides). The percentage of patients with episodes of acute cellular rejection requiring treatment (steroid bolus or increased immunosuppression) previous to the study biopsy was 14.8% (4/27) in the HAS 3 group compared with 10.5% (15/143) in the HAS 1 to 2 groups ($P = 0.75$).

Comparison With Existing Grading System

In our series, the HAS system was superior to currently used grading systems in predicting graft survival in the setting of post-LT recurrent hepatitis C, particularly in the severe end of the grading spectrum (Fig. 6). In univariable survival analysis, a higher HR (7.8; 95% CI, 4.4-13.7) was demonstrated for the HAS classification compared with conventional grading systems at optimal cutoff scores in predicting graft loss. Finally, the HAS classification was the only system that was predictive of time-dependent graft loss in the final multivariable model, which included grade and stage by all conventional systems (Table 4).

Assessment of Histopathologic Criteria for FCH-C According to ITS Consensus[12]

We have confirmed and further characterized the diagnostic/prognostic value of some of the FCH-associated histologic findings included in the ITS consensus criteria, namely ductular reaction and cholestasis. Similarly, hepatocyte ballooning represented an important histologic feature in this setting, but we have not confirmed the predominant centrilobular localization of this finding in our series. Among 48 patients showing prominent hepatocyte ballooning in the entire population, the percentage showing ballooning in a predominantly centrilobular distribution was 11.5% (3/26) in FCH-C patients compared with 9% (2/22) in non-FCH-C patients ($P = 0.84$). With regard to the "paucity of inflammation" criterion, the median grade of portal, interface, and lobular inflammation according to the HAI system was 2 (1 to 3), 2 (1 to 3), and 2 (1 to), respectively, in FCH-C patients (HAS 3) compared with 1 (0 to 4), 2 (0 to 4), and 2 (0 to 4) ($P = 0.82$) in non-FCH-C patients (HAS 1 to 2). Similar results were obtained upon comparison with other grading systems (data not shown). Therefore, our data suggest that all histologic components of necroinflammation in cases of FCH-C are comparable with

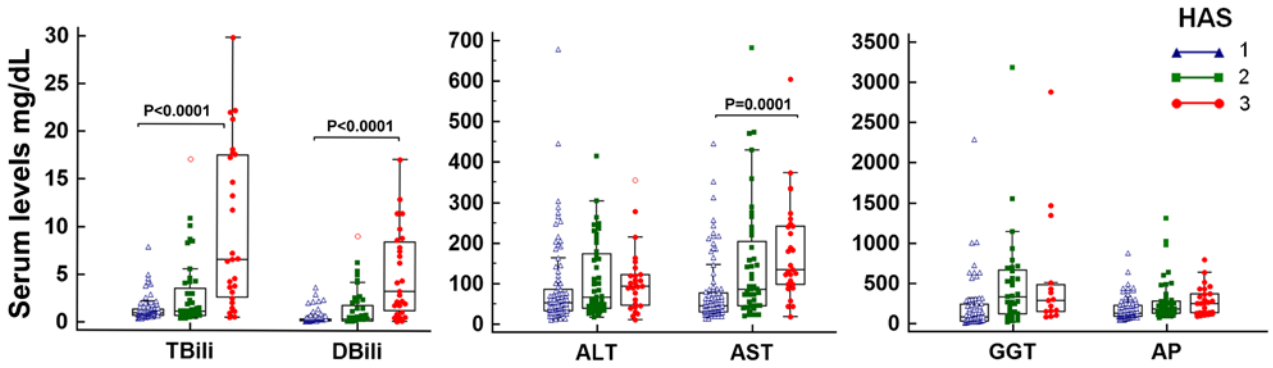

FIGURE 5. Bilirubin and liver enzyme levels at the time of index biopsy according to HAS classification. Statistically significant difference in total and direct bilirubin levels is observed between HAS 1 versus HAS 3 groups, in keeping with the severe cholestatic liver disease generally seen in patients classified as HAS 3. ALT indicates alanine aminotranferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; DBili, direct bilirubin; GGT, γ-glutamyltransferase; TBili, total bilirubin.

those seen in non-FCH recurrent hepatitis C and that a predominantly centrilobular distribution of hepatocyte ballooning is probably not diagnostically useful.

DISCUSSION

With increasing success of new immunosuppression regimens in controlling rejection-related graft loss, which has become uncommon in most centers,[28–30] efforts have now shifted to attempting to monitor and prevent the progressive liver disease associated with recurrent HCV infection, which currently represents a leading cause of allograft loss and patient mortality in this population.[6–8,13,14,16,18] Although differences exist among the various currently used hepatitis grading systems, they tend to yield comparable results as prognostic tools, as similar histologic features (a combination of portal, interface, and lobular necroinflammatory activity) are evaluated. However, in addition to necroinflammatory activity, other histopathologic features are well described in the context of post-LT recurrent hepatitis C, particularly in severe, cholestatic forms.

Ductular reaction—a relatively nonspecific finding that may be seen in a variety of liver diseases and that may be particularly prominent in biliary obstructive processes—for instance, is also described in chronic hepatitis of various etiologies, including chronic viral hepatitis C.[31–33] Irrespective of its etiology, ductular reaction is now

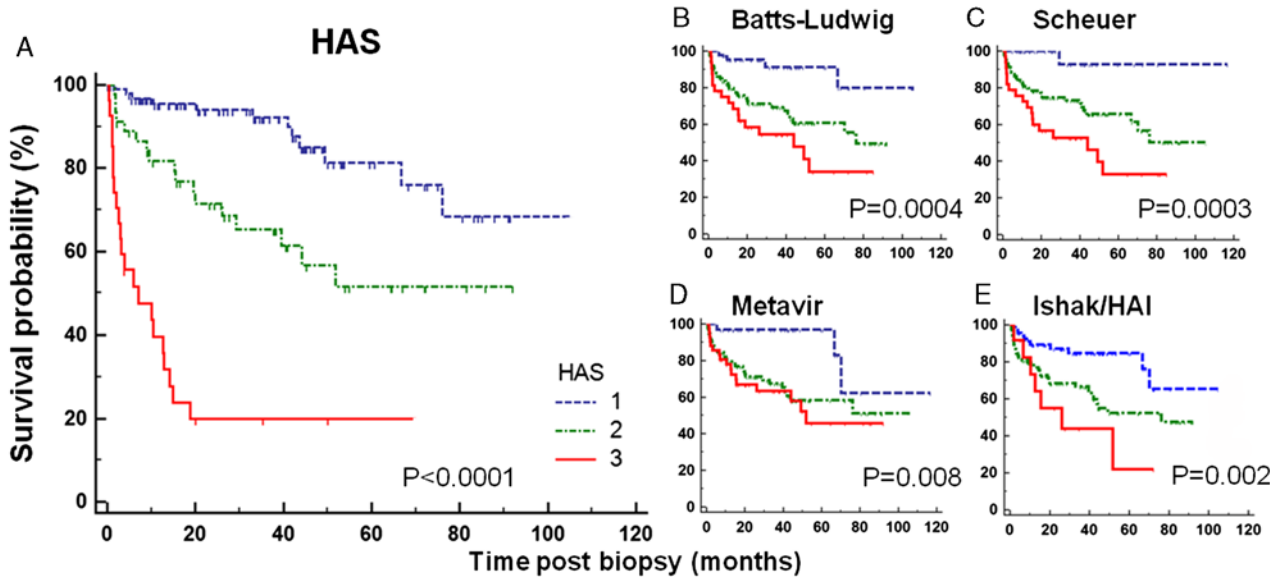

FIGURE 6. Comparison between the HAS classification (A) and other currently used hepatitis grading systems (B–E) in predicting graft loss in the setting of recurrent hepatitis C. Whereas all grading systems show prognostic utility, the HAS classification more accurately identifies the patient group at highest risk for graft loss (ie, HAS 3 or FCH-C). B–E, Blue dotted line, minimal activity (Batts-Ludwig grades 0 to 1, Scheuer grades 0 to 1, Ishak scores 0 to 4, and Metavir A0); green dotted line, mild activity (Batts-Ludwig grade 2, Scheuer grade 2, HAI scores 5 to 8, and Metavir A1); and red solid line, moderate to severe activity (Batts-Ludwig grades 3 to 4, Scheuer grades 3 to 4, HAI score >8, and Metavir A2 to 3). Kaplan-Meier survival analysis and log rank test.

TABLE 4.

Predictor	HR	95% CI	*P*
Univariable analysis			
HAS			
3 vs. 1-2	7.8	4.4-13.7	<0.001
2 vs. 1	3.2	1.5-6.8	0.002
3 vs. 1	13.2	6.4-26.9	<0.001
Necroinflammatory activity			
Batts-Ludwig (2-4 vs. 0-1)	5.1	1.8-14.1	0.002
Scheuer (3-4 vs. 0-2)	2.4	1.4-4.3	0.003
Metavir (2-3 vs. 0-1)	5.0	1.6-16.4	0.006
HAI/Ishak (2-4 vs. 0-1)	2.8	1.4-5.5	0.002
Moderate-severe necroinflammation*	1.7	0.9-3.0	0.062
Fibrosis stage			
Batts-Ludwig (2-4 vs. 0-1)	3.5	1.9-6.3	<0.001
Scheuer (2-4 vs. 0-1)	3.5	1.9-6.3	<0.001
Metavir (2-3 vs. 0-1)	3.5	1.9-6.3	<0.001
HAI (3-6 vs. 0-2)	2.7	1.6-4.7	<0.001
Moderate-severe fibrosis*	3.5	1.9-6.3	<0.001
Multivariable analysis			
HAS			
3 vs. 1-2	5.5	2.9-10.7	<0.001
2 vs. 1	3.5	1.5-8.3	0.004
3 vs. 1	14.5	5.5-38.3	<0.001
Moderate-severe fibrosis*	1.85	0.9-3.8	0.09
Moderate-severe necroinflammation*	1.02	0.5-1.9	0.94

*Refers to scores greater than the optimal cutoff point (indicated in parentheses) for predicting graft loss by at least 1 of the "conventional" grading/staging systems.

CI indicates confidence interval; HAI, Hepatitis Activity Index; HR, hazard ratio; HAS, Hepatitis Aggressiveness Score.

thought to reflect hepatic progenitor cell activation,[34] triggered as an "alternative" pathway of liver parenchymal regeneration, often occurring in the setting of parenchymal replicative arrest and hepatocellular senescence.[32,35,36] In chronic hepatitis C patients, ductular reaction seems to be more common and pronounced in allograft compared with native livers[8] and, in cases of FCH-C, may be particularly exuberant, closely mimicking biliary obstruction. Prominent ductular reaction was almost invariably present in the cases classified as FCH-C/HAS 3 in our series but was also seen in non-FCH-C patients and represented an important predictor of graft survival in our study population. Recent evidence suggests that even milder degrees of ductular reaction may have prognostic implications in this setting. Meriden et al,[37] in a large retrospective study of recurrent hepatitis C patients, have shown significantly greater cytokeratin 19 expression (reflecting the magnitude of ductular reaction and hepatic progenitor cell activation) by immunohistochemistry in the "rapid fibrosers" (defined as METAVIR stage F3-F4 within 2 y of LT) than in the "slow fibrosers" (defined as METAVIR stage F0-F1 within 2 y of LT) subgroups.

Likewise, hepatocyte ballooning—characterized by enlarged, swollen, rounded hepatocytes showing cytoplasmic rarefaction and reticulation—is seen in a variety of liver diseases with hepatocyte injury. Although the pathogenesis of the hepatocyte ballooning seen in chronic viral hepatitis may differ from that seen in steatohepatitis and cholestatic diseases,[38] the latter is often a prominent feature in patients with FCH-C. In this setting, some authors postulate a direct HCV cytopathic effect as the underlying etiology of this histologic finding.[21,23] In contrast, cholestasis, either canalicular or intracellular, is not part of the histopathologic spectrum of "uncomplicated" chronic hepatitis C in either native livers or allografts. Its presence in this context signifies either a superimposed condition, such as biliary obstruction, rejection, drug toxicity, or development of a cholestatic form of hepatitis C. In the latter scenario, the precise mechanism leading to cholestasis is largely unknown. Finally, the periportal sinusoidal/pericellular fibrosis in association with ductular reaction is a distinctive pattern of fibrosis that was first described in the context of FCH-B.[23,39]

The main focus of this study is to introduce and assess the utility of a novel approach to histopathologic grading of post-LT recurrent hepatitis C based exclusively on FCH features. Although particularly common and prominent in FCH-C, most of the aforementioned features are also seen in non-FCH recurrent hepatitis C in various combinations and, therefore, reflect a spectrum of histopathologic changes in this population rather than findings restricted to severe, cholestatic variants. We believe our proposed approach is feasible, as it utilizes features that are familiar to pathologists and assessed routinely during examination of liver biopsy specimens, and yields valuable prognostic information, including risk of fibrosis progression and graft loss. In addition, we have defined all subgroups in this study based exclusively on histopathologic/morphologic criteria (except for exclusion of biliary obstruction and vascular complications) to more specifically evaluate the role of histopathology in the characterization of FCH-C.

Strengths of this study include the relatively large number of patients, including one of the largest groups of FCH-C cases reported to date, long median follow-up time, correlation with clinical parameters and outcome data, and side-by-side comparison with all of the commonly used hepatitis grading systems. In addition, in contrast to all other grading schemes, our proposed system utilizes features other than inflammatory activity and, therefore, offers a different perspective on the histologic evaluation of recurrent hepatitis C. As part of being a novel approach to grading of recurrent hepatitis C, however, the reproducibility of histopathologic findings and the prognostic value of the HAS system, or modifications thereof, need to be confirmed in prospective studies and by other investigators. The value of this novel approach must also be evaluated outside of the LT setting, especially in HCV-infected patients at highest risk for development of severe cholestatic forms of the disease (including human immunodeficiency virus coinfection, nonliver solid organ transplantation, bone marrow transplantation, etc.).

Despite these limitations, we believe that our data strongly support the contention that the utilization of FCH-C histopathologic features as the basis of an alternative grading system for recurrent hepatitis C may represent a valuable addition to the currently available repertoire of hepatitis grading systems. When at least 3 of

the 4 FCH features are present on a liver biopsy sample, in the appropriate clinical setting [ie, absence of biliary obstruction and hepatic artery thrombosis, as per current (ITS) consensus recommendations, as described in the Patients and Methods section] patients almost invariably experience severe cholestatic liver disease with rapid progression of liver fibrosis, generally leading to graft failure and/or death within several months (ie, consistent with a clinicopathologic diagnosis of FCH-C). Patients showing fewer FCH features have variable, but on average significantly less, aggressive forms of recurrent hepatitis C. Therefore, our data clearly point to a more precise histopathologic characterization of FCH-C (ie, ≥ 3 of 4 histologic criteria) than what is currently available in the literature (Table 1).

Besides aiding in the identification and histopathologic characterization of FCH-C, our proposed system was also able to accurately stratify non-FCH-C patients into distinct prognostic categories (nonaggressive and aggressive hepatitis) on the basis of the number of FCH features present on liver biopsy samples, therefore representing a useful grading system for the entire population of recurrent hepatitis C patients. Although all conventional grading systems included in this study were of prognostic value, the HAS system was superior to all others in predicting graft loss in our population. These results must still be validated by prospective studies.

In addition, several studies have reported a faster rate of fibrosis progression in HCV-positive patients transplanted in recent years compared with older series,[37,40–42,4] and some authors have drawn attention to the absence of a corresponding increase in necroinflammatory activity, suggesting the existence of inflammation-independent mechanisms of fibrosis progression in this setting.[37,42] To this end, in our study population, the HAS classification remained highly predictive of patient and graft survival independently of necroinflammatory activity by any of the currently utilized grading systems, suggesting that at least some of the included histologic features may represent morphologic markers of putative inflammation-independent factors.

Our study confirmed the prognostic value of several previously described FCH features but failed to prove the utility of some of the histopathologic findings included in the current diagnostic criteria for FCH-C, as suggested by the ITS consensus—that is, hepatocyte ballooning in a centrilobular distribution (as opposed to any distribution) and paucity of inflammation. Although characteristic of hepatitis B-associated FCH, the prevalence of the latter findings in HCV-associated cases has been debated.[23,43] In our series, either feature was only present in a minority of FCH-C cases, as defined in this study. In fact, the average portal, interface, and lobular inflammatory scores were similar in FCH-C and non-FCH-C cases in our study population, whereas hepatocyte ballooning present exclusively in centrilobular areas (or showing obvious centrilobular accentuation) was observed in a small minority of cases in both groups. In most cases, hepatocyte ballooning was diffuse and, in some cases, predominantly periportal. Our data, therefore, do not support the aforementioned features as part of the histopathologic characterization of HCV-associated FCH.

CONCLUSIONS

In this study, we have introduced a novel approach to the histologic grading of posttransplantation recurrent hepatitis C based exclusively on FCH histologic features. The features evaluated in the HAS classification are distinct from those used in existing grading systems and reflect pathologic processes that may be important in the pathogenesis of recurrent hepatitis C after LT, particularly in severe forms. Our proposed grading system allows accurate identification of FCH-C cases and stratification of all recurrent hepatitis C patients into categories that were highly predictive of graft loss independently of necroinflammation or stage. We believe our proposed classification system represents a valuable tool to be used in the setting of post-LT recurrent hepatitis C.

REFERENCES

1. Thuluvath PJ, Guidinger MK, Fung JJ, et al. Liver Transplantation in the United States, 1999–2008. *Am J Transpl*. 2010;10(pt 2): 1003–1019.
2. Garcia-Retortillo M, Forns X, Feliu A, et al. Hepatitis C virus kinetics during and immediately after liver transplantation. *Hepatology*. 2002;35:680–687.
3. Gane EJ, Portmann BC, Naoumov NV, et al. Long-term outcome of hepatitis C infection after liver transplantation. *N Engl J Med*. 1996;334:815–820.
4. Berenguer M, Ferrell L, Watson J, et al. HCV-related fibrosis progression following liver transplantation: increase in recent years. *J Hepatol*. 2000;32:673–684.
5. Ramírez S, Pérez-Del-Pulgar S, Forns X. Virology and pathogenesis of hepatitis C virus recurrence. *Liver Transpl*. 2008;14(suppl 2): S27–S35.
6. Berenguer M. Natural history of recurrent hepatitis C. *Liver Transpl*. 2002;8(suppl 1):S14–S18.
7. Belli LS, Burroughs AK, Burra P, et al. Liver transplantation for HCV cirrhosis: improved survival in recent years and increased severity of recurrent disease in female recipients: results of a long term retrospective study. *Liver Transpl*. 2007;13:733–740.
8. Demetris AJ. Evolution of hepatitis C virus in liver allografts. *Liver Transpl*. 2009;15(suppl 2):S35–S41.
9. Charlton M, Seaberg E. Impact of immunosuppression and acute rejection on recurrence of hepatitis C: results of the National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. *Liver Transpl Surg*. 1999;5(suppl 1): S107–S114.
10. Satapathy SK, Sclair S, Fiel MI, et al. Clinical characterization of patients developing histologically-proven fibrosing cholestatic hepatitis C post-liver transplantation. *Hepatol Res*. 2011;41:328–339.
11. Demetris AJ, Eghtesad B, Marcos A, et al. Recurrent hepatitis C in liver allografts: prospective assessment of diagnostic accuracy, identification of pitfalls, and observations about pathogenesis. *Am J Surg Pathol*. 2004;28:658–669.
12. Wiesner RH, Sorrell M, Villamil F. Report of the first International Liver Transplantation Society expert panel consensus conference on liver transplantation and hepatitis C. *Liver Transpl*. 2003;9:S1–S9.
13. Prieto M, Berenguer M, Rayón JM, et al. High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. *Hepatology*. 1999;29:250–256.
14. Firpi RJ, Abdelmalek MF, Soldevila-Pico C, et al. One-year protocol liver biopsy can stratify fibrosis progression in liver transplant recipients with recurrent hepatitis C infection. *Liver Transpl*. 2004;10:1240–1247.

15. Yilmaz N, Shiffman ML, Stravitz RT, et al. A prospective evaluation of fibrosis progression in patients with recurrent hepatitis C virus following liver transplantation. *Liver Transpl*. 2007;13: 975–983.
16. Burra P. Hepatitis C. *Semin Liver Dis*. 2009;29:53–65.
17. Watt K, Veldt B, Charlton M. A practical guide to the management of HCV infection following liver transplantation. *Am J Transpl*. 2009;9:1707–1713.
18. Brown RS. Hepatitis C and liver transplantation. *Nature*. 2005;436:973–978.
19. Taga SA, Washington MK, Terrault N, et al. Cholestatic hepatitis C in liver allografts. *Liver Transpl Surg*. 1998;4:304–310.
20. Dixon LR, Crawford JM. Early histologic changes in fibrosing cholestatic hepatitis C. *Liver Transpl*. 2007;13:219–226.
21. McCaughan GW, Bowen DG. Pathogenesis of cholestatic hepatitis C. *J Hepatol*. 2011;54:392–394.
22. Narang TK, Ahrens W, Russo MW. Post-liver transplant cholestatic hepatitis C: a systematic review of clinical and pathological findings and application of consensus criteria. *Liver Transpl*. 2010;16:1228–1235.
23. Xiao S-Y, Lu L, Wang HL. Fibrosing cholestatic hepatitis: clinicopathologic spectrum, diagnosis and pathogenesis. *Int J Clin Exp Pathol*. 2008;1:396–402.
24. Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol*. 1995;19:1409–1417.
25. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol*. 1995;22:696–699.
26. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol*. 1991;13:372–374.
27. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology*. 1996;24:289–293.
28. Neil DAH, Hübscher SG. Current views on rejection pathology in liver transplantation. *Transpl Int*. 2010;23:971–983.
29. Neuberger J. Incidence, timing, and risk factors for acute and chronic rejection. *Liver Transpl Surg*. 1999;5(suppl 1):S30–S36.
30. Hübscher SG. What is the long-term outcome of the liver allograft? *J Hepatol*. 2011;55:702–717.
31. Libbrecht L, Desmet V, Van Damme B, et al. Deep intralobular extension of human hepatic "progenitor cells" correlates with parenchymal inflammation in chronic viral hepatitis: can "progenitor cells" migrate? *J Pathol*. 2000;192:373–378.
32. Clouston AD, Powell EE, Walsh MJ, et al. Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. *Hepatology*. 2005;41:809–818.
33. Delladetsima J, Alexandrou P, Giaslakiotis K, et al. Hepatic progenitor cells in chronic hepatitis C: a phenomenon of older age and advanced liver disease. *Virchows Arch*. 2010;457:457–466.
34. Roskams TA, Theise ND, Balabaud C, et al. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology*. 2004;39:1739–1745.
35. Lunz JG III, Tsuji H, Nozaki I, et al. An inhibitor of cyclin-dependent kinase, stress-induced p21Waf-1/Cip-1, mediates hepatocyte mito-inhibition during the evolution of cirrhosis. *Hepatology*. 2005;41:1262–1271.
36. Sekoguchi S, Nakajima T, Moriguchi M, et al. Role of cell-cycle turnover and oxidative stress in telomere shortening and cellular senescence in patients with chronic hepatitis C. *J Gastroenterol Hepatol*. 2007;22:182–190.
37. Meriden Z, Forde KA, Pasha TL, et al. Histologic predictors of fibrosis progression in liver allografts in patients with hepatitis C virus infection. *Clin Gastroenterol Hepatol*. 2010;8:289–296, 296.e1–296.e8.
38. Lackner C, Gogg-Kamerer M, Zatloukal K, et al. Ballooned hepatocytes in steatohepatitis: the value of keratin immunohistochemistry for diagnosis. *J Hepatol*. 2008;48:821–828.
39. Davies SE, Portmann BC, O'Grady JG, et al. Hepatic histological findings after transplantation for chronic hepatitis B virus infection, including a unique pattern of fibrosing cholestatic hepatitis. *Hepatology*. 1991;13:150–157.
40. Berenguer M, Prieto M, San Juan F, et al. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. *Hepatology*. 2002;36:202–210.
41. Burak KW, Kremers WK, Batts KP, et al. Impact of cytomegalovirus infection, year of transplantation, and donor age on outcomes after liver transplantation for hepatitis C. *Liver Transpl*. 2002;8:362–369.
42. Baiocchi L, Angelico M, Petrolati A, et al. Correlation between liver fibrosis and inflammation in patients transplanted for HCV liver disease. *Am J Transplant*. 2008;8:673–678.
43. Denk H. What is expected from the pathologist in the diagnosis of viral hepatitis? *Virchows Arch*. 2011;458:377–392.

