TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Interactions between virus-related factors and post-transplant ascites in patients with hepatitis C and no cirrhosis: role of cryoglobulinemia

Simona Tripon,[1] Claire Francoz,[1,2] Anna Albuquerque,[3] Valérie Paradis,[2,3] Hamza Boudjema,[1] Hélène Voitot,[4] Jacques Belghiti,[5] Dominique Valla[1,2] and François Durand[1,2]

1 Hepatology and Liver Intensive Care, Hospital Beaujon, Clichy, France
2 CRI, UMR 1149, INSERM, Paris Diderot University, Paris, France
3 Pathology Department, Hospital Beaujon, Clichy, France
4 Biochemistry and Molecular Genetics, Hospital Beaujon, Clichy, France
5 Hepatobiliary and Pancreatic Surgery and Liver Transplantation, Hospital Beaujon, Clichy, France

Keywords
ascites, cryoglobulinemia, hepatitis C, liver transplantation.

Correspondence
Claire Francoz, Service d'Hépatologie, Hôpital Beaujon, 100 Boulevard du Général Leclerc, 92110, Clichy, France.
Tel.: +33 1 40 87 50 91;
fax: +33 1 40 87 44 55;
e-mail: claire.francoz@bjn.aphp.fr

Conflicts of interest
The authors have declared no conflicts of interest.

Received: 15 April 2014
Revision requested: 2 June 2014
Accepted: 21 September 2014
Published online: 19 November 2014

doi:10.1111/tri.12466

Summary

Refractory ascites may appear in liver transplant recipients with recurrence of hepatitis C virus infection, even in the absence of advanced fibrosis. The mechanisms are unclear. The aim was to determine whether post-transplant cryoglobulinemia could be a predisposing factor for ascites in this population. Retrospective data of 82 liver transplant recipients with HCV recurrence surviving more than 1 year were collected. Cryoglobulinemia was systematically tested after transplantation. All patients had 1-year protocol biopsy with assessment of sinusoidal distension, perisinusoidal fibrosis, and centrolobular necrosis. Additional biopsies were performed when needed. Fourteen of 82 patients (17%) developed refractory ascites. When ascites appeared, fibrosis was stage F0–F1 in 36% and F2–F3 in 57%. Factors independently associated with post-transplant ascites were pretransplant refractory ascites ($P = 0.001$), fibrosis ≥stage 2 at 1 year ($P = 0.002$), perisinusoidal fibrosis at 1 year ($P = 0.02$), and positive cryoglobulinemia ($P = 0.02$). Patients with ascites had a significantly worse prognosis compared to those without ascites. Refractory ascites may occur in liver transplant recipients with HCV recurrence in the absence of advanced fibrosis. The finding that both positive cryoglobulinemia and perisinusoidal fibrosis at 1 year were significantly associated with ascites suggests that liver microangiopathy is involved in the mechanisms of HCV-related ascites.

Introduction

Hepatitis C virus (HCV)-related cirrhosis, with or without hepatocellular carcinoma (HCC) is one of the most common indications for liver transplantation in Western countries. The vast majority of candidates for transplantation are still positive for HCV-RNA as antiviral therapy has been ineffective or contraindicated prior to transplantation. As a result, post-transplant recurrence occurred in the majority of patients transplanted over the last decades. The progression of recurrent hepatitis C is markedly accelerated by immunosuppression [1–3] with a deleterious impact on the outcome compared to liver transplant recipients without disease recurrence [4,5]. For instance, in a large series, 5-year post-transplant survival was found to be 61% in HCV-positive patients compared to 76% in HCV-negative patients, the difference being statistically significant [4].

A particular feature of post-transplant HCV recurrence is that in a subgroup of patients, ascites develops even in the absence of advanced fibrosis [6]. This profile is clearly different from that of nontransplanted HCV-infected patients where refractory ascites only occurs at a stage of

advanced cirrhosis. Ascites is one of the complications that define decompensated cirrhosis [7]. By contrast, in transplanted patients with HCV recurrence, ascites may occur at an earlier stage of fibrosis (fibrosis stage 0 to 2), without evidence of hepatic vein outflow obstruction and with normal hepatic venous pressure gradient [6, 8–10]. This feature is not observed in non-HCV-infected liver transplant recipients. In patients with post-transplant HCV recurrence, female gender, prolonged cold ischemia time, and high serum creatinine were found to be independent predictors of refractory ascites [6]. However, no specific parenchymal or vascular profiles on histopathology have been documented. Importantly, post-transplant survival was significantly lower in patients with refractory ascites compared to those without ascites [6,9]. Even though predisposing factors have been identified, the pathogenesis of refractory ascites in HCV-infected recipients remains unclear, and more data are needed. The aim of this study was to determine whether post-transplant cryoglobulinemia, a possible cause of microcirculatory changes, could be involved in post-transplant HCV-related ascites.

Patients and methods

Study population

From January 2000 to June 2010, 647 first elective liver transplantations were performed in our institution, among which 200 in HCV-infected patients (31%). Among these 200 patients, 118 were excluded due to the following reasons: right lobe living donor liver transplantation ($n = 25$), death within the first year post-transplantation ($n = 33$), no available data on cryoglobulinemia after transplantation ($n = 46$), loss of follow-up ($n = 4$), and absence of HCV recurrence after transplantation (undetectable HCV-RNA both immediately prior to and in the long term after transplantation, $n = 10$). The study population consisted of the remaining 82 consecutive patients. Fifty three of these 82 patients (65%) had received antiviral therapy prior to transplantation but 96% were still HCV-RNA positive. Only three patients who were HCV-RNA negative immediately prior to transplantation had HCV recurrence after transplantation. Sixty seven were transplanted with a whole liver graft, and 15 (18%) had split liver transplantation. The study was approved by the local ethics committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Five patients who had impaired renal function postoperatively received induction immunosuppression with basiliximab, mycophenolate mofetil, and steroids. In this subgroup, introduction of calcineurin inhibitors (CNis) was delayed up to 7 days postoperatively. None of the remaining patients received induction immunosuppression. Maintenance immunosuppression consisted of a combination of CNIs (tacrolimus in 79 and cyclosporine in 2) and mycophenolate mofetil in 67 patients. One patient received a combination of sirolimus and mycophenolate mofetil. After 1-year post-transplantation, target trough levels were 3–5 ng/ml for tacrolimus, 50–100 ng/ml for cyclosporine, and 5–8 ng/ml for sirolimus.

Diagnosis and management of recurrent HCV infection

In all patients, recurrence of HCV infection was documented by post-transplant serum HCV-RNA positivity as well as biochemical and pathological features consistent with recurrent hepatitis. HCV genotype was 1 in 58%, 2 in 4%, 3 in 20%, 4 in 14%, and 5 in 4%. Protocol liver biopsies were performed 1-year post-transplantation and thereafter, either 2 or 3 years after transplantation depending upon the findings of the 1-year protocol biopsy and/or antiviral therapy. Independent of protocol biopsies, transvenous biopsy was systematically performed in patients experiencing ascites (provided a protocol biopsy had not been performed <1 year before the occurrence of ascites). In those with ascites requiring additional biopsy, transvenous route was used due to the increased risk of bleeding with transcapsular biopsy. For this study, all biopsies were examined by two experienced pathologists (A.A. and V.P.) who were not aware of the clinical context. Fibrosis and activity were graded according to the METAVIR score, as previously described [11]. In addition, sinusoidal distension, perisinusoidal fibrosis, and centrolobular necrosis were categorized as "present" or "absent" but were not graded.

Antiviral therapy was systematically considered in patients with a METAVIR fibrosis score of 2 or more and no contraindication to therapy. In the whole series, antiviral therapy consisted of a combination of pegylated interferon alfa-2a and ribavirin. Target doses were 180 μg/week subcutaneously for pegylated interferon alfa-2a and 1000 mg/day for ribavirin. Doses were adjusted according to drug-related side effects. Whenever possible, the total duration of antiviral therapy was 48 weeks [12]. Sustained virological response was defined as undetectable serum HCV-RNA at the end of a 24-week untreated follow-up period [13].

Other variables

During the study period, serum cryoglobulin was systematically tested in all patients at least once during outpatient follow-up, 6 month or more after transplantation. Serum cryoglobulinemia was studied twice in 26 patients and thrice in 5. Mean time from transplantation to first testing for cryoglobulinemia was 43 ± 33 months (range 6–139 months). Cryoglobulinemia was initially tested in patients with post-transplantation ascites then we decided

to test all the patients in a second period. Venous blood was collected and placed in prewarmed equipment and transported at 37 °C to the laboratory. After clotting and centrifugation at 37 °C, serum was removed and incubated at 20 °C and 4 °C for 24 h. Serum samples were then examined for cryoprecipitation. Positive cases were defined by the presence of a cryoprecipitate at the bottom of the test tube [14]. Among patients who have been tested twice, five had discordant results and cryoglobulinemia was considered positive. In contrast, in those who were tested thrice, all results were concordant.

Pre- and post-transplant diabetes was defined by the need for insulin and/or oral antidiabetic agents to achieve glycemic control.

A diagnosis of refractory ascites was based on the International Ascites Club criteria [15]. Patients were considered to have post-transplant refractory ascites when they had either diuretic-resistant or diuretic-intractable ascites. Diuretic-resistant ascites corresponded to persistent ascites despite optimal diuretic therapy including furosemide and/or spironolactone and dietary sodium restriction. Diuretic-intractable ascites corresponded to the impossibility to achieve optimal diuretic doses due to the occurrence of metabolic changes or impaired renal function (e.g., severe hyponatremia and/or increase in serum creatinine).

Doppler ultrasound was routinely performed at least every 6 months following the first year after transplantation. Hepatic artery resistive index and systolic acceleration time (s) were specifically measured when post-transplant ascites appeared.

Statistical analysis

Continuous variables were expressed as means ± SD. Categorical variables were expressed as percentages and were grouped if needed. Predictive analysis concerned the predefined endpoint of post-transplant refractory ascites. Univariate analysis was performed using Mann–Whitney or Fisher's exact tests. Multivariate analysis was performed using logistic regression with a descending selection and a significance level of 0.05. Odds ratios were computed with their 95% confidence intervals. Survival analysis was performed using the Kaplan–Meier method, and comparisons were performed using the log-rank test. Statistical analysis was performed using the SAS statistical software (version 9.3; SAS® institute, Cary, NC).

Results

Recurrence of HCV infection and post-transplant management

According to the inclusion criteria, all patients had HCV recurrence and all were found to be positive for serum HCV-RNA within the first year after transplantation. Protocol biopsy at 1 year showed fibrosis staged F0, F1, F2, and F3 in 32%, 45%, 19%, and 3%, respectively. A single patient had cirrhosis (F4) at 1 year. During follow-up, 24 patients (29%) received antiviral therapy. Mean interval between transplantation and the initiation of antiviral therapy was of 25 ± 20 months (range, 1.5–77 months). Mean duration of antiviral therapy was 42 ± 20 weeks (range, 11–96 weeks). Only 11 of the 24 (46%) treated patients received antiviral therapy for at least 48 weeks. In the remaining 13 patients (54%), antiviral therapy was discontinued due to the absence of efficacy (<2 $\log_{10}$ UI/ml decrease in HCV-RNA level from baseline at 12 weeks of therapy) and/or serious adverse events, according to the current recommendations [16]. Nine of the 24 treated patients (37%) had virological response, of whom, six had received antiviral therapy for 48 weeks or more. At the end of follow-up, 7 of 24 treated patients (29%) had sustained virological response, as defined above. In the remaining patients, mean viral load at the end of follow-up was 6.5 ± 7.7 $\log_{10}$ UI/ml. At the end of follow-up, 13% had documented cirrhosis (47 ± 29 months on average post-transplantation). Two patients were retransplanted due to recurrence of HCV infection.

Concomitant post-transplant events

Six patients experienced biopsy-proven acute rejection within the first 3 months and four required steroid boluses. All were responsive to steroid boluses and none of the patients developed chronic rejection. Similarly, none of the patients in this series experienced delayed acute rejection.

Five patients had biliary complications (anastomotic biliary strictures). These complications were successfully treated by biliary reconstruction (hepaticojejunostomy) in three patients and endoscopic management in the other two. Three patients had anastomotic hepatic artery stenosis. Two patients underwent percutaneous angioplasty and stenting. The remaining patient did not have angioplasty because stenosis was considered nonhemodynamically significant. None of the patients developed diffuse ischemic cholangiopathy. None of the patients in this series had cutaneous changes consistent with cryoglobulinemia.

Post-transplant refractory ascites: predisposing factors

During the study period, 14 of 82 patients with HCV recurrence (17%) developed refractory ascites, as defined above. Median time between transplantation and the occurrence of ascites was 14 months (range, 4 to 92 months). Median interval between the occurrence of refractory ascites and the liver biopsy was 3 months (range, 0.5 to 8 months). None of the patients who developed ascites had alcohol

abuse. Doppler ultrasound was systematically performed showing hepatopetal portal flow in all cases. The size of the spleen was 14.2 ± 3.2 cm. Transthoracic echocardiography was systematically performed, and none of the patients had evidence of myocardial dysfunction. In patients with refractory ascites, the biopsy showed fibrosis graded F0, F1, F2, F3, and F4 in 1, 4, 5, 3, and 1 patient, respectively. Almost all patients did not have cirrhosis when ascites occurred, showing that, even in the absence of cirrhosis, patients with HCV recurrence could develop ascites. Among pathological features, perisinusoidal fibrosis but not sinusoidal distension was significantly associated with refractory ascites (Table 2). Protein concentration in the ascitic fluid was quite variable (average: 17.5 ± 9 g/l with a range of 8 to 40 g/l). Large volume paracentesis was needed between once a week and a maximum of once a month according to the patients.

Among the 14 patients with post-transplant refractory ascites, five received antiviral therapy. Antiviral therapy had been started before the occurrence of refractory ascites (10 months) in one patient, at the time of ascites in two patients and after the occurrence of ascites in the remaining two patients (6 and 20 months, respectively). Only one of these five patients had sustained virological response, which was followed by resolution of ascites.

There was no correlation between vascular complications, which were uncommon in this population, and post-transplant refractory ascites. Hepatic artery indexes on Doppler ultrasound were similar in both groups (Table 3). None of the patients had evidence of hepatic outflow obstruction on Doppler ultrasound such as undetectable blood flow in the hepatic veins, weak measurable flow (<10 cm/s) or monophasic waveform [17].

Among the 14 patients with post-transplant refractory ascites, nine patients who did not have protocol biopsy within 1 year before the occurrence of ascites had transjugular liver biopsy with direct measurement of hepatic venous pressure gradient (HVPG). In these patients, HVPG was of 11 ± 4 mmHg (range 5 to 17 mmHg). Four patients had HVPG lower than 10 mmHg.

In univariate analysis, pretransplantation refractory ascites, donor age, post-transplant diabetes, fibrosis staged 2 or more and the presence of perisinusoidal fibrosis on 1-year post-transplant biopsy and positive cryoglobulinemia were significantly associated with post-transplant refractory ascites (Tables 1, 2, and 3). In multivariate analysis, pretransplant refractory ascites, fibrosis staged 2 or more on 1-year post-transplant biopsy, presence of perisinusoidal fibrosis on 1-year post-transplant biopsy, and positive cryoglobulinemia were significantly and independently associated with post-transplant ascites (Table 4). As shown in Table 3, not all patients who were positive for cryoglobulins developed post-transplant ascites. Interestingly, among the group of patients who were positive for cryoglobulins,

Table 1. Main characteristics of the study population.

Variables	All patients (n = 82)	With ascites (n = 14)	Without ascites (n = 68)	P value*
Recipients characteristics at listing				
Age (years)	52 ± 7	51 ± 7	52 ± 8	0.56
Gender (%female)	27	36	25	0.17
Body mass index	25 ± 5	26 ± 8	25 ± 5	0.42
Diabetes (%)	17	29	15	0.13
HCV-RNA positive (%)	96	100	96	0.56
Pretransplant refractory ascites (%)	21	43	16	0.02
Bilirubin (μmol/l)	59 ± 75	92 ± 135	52 ± 55	0.07
International normalized ratio	1.6 ± 0.7	1.6 ± 0.8	1.6 ± 0.7	0.7
Creatinine (μmol/l)	87 ± 66	95 ± 60	85 ± 68	0.61
MELD score	15 ± 7	17 ± 9	15 ± 6	0.33
MELD score in those without HCC	18 ± 7	19 ± 9	18 ± 6	0.68
Albumin (g/l)	37 ± 37	28 ± 8	38 ± 41	0.35
Platelets ($\times 10^9$/l)	90 ± 57	85 ± 64	91 ± 56	0.7
Donor characteristics				
Age (years)	51 ± 18	61 ± 17	48 ± 18	0.02
Gender (%female)	41	28	44	0.13
Body mass index	25 ± 5	25 ± 4	25 ± 5	0.66
Macrovesicular steatosis >30% (%)	19	36	16	0.07
Transplant factors				
Split liver (%)	15	16	7	0.25
Cold ischemia time (min)	501 ± 155	450 ± 113	511 ± 161	0.18
Warm ischemia time (min)	56 ± 16	50 ± 14	57 ± 16	0.16

*P value between patients with and without ascites.

Table 2. Post-transplant pathological features on 1-year protocol biopsy according to the presence or absence of refractory ascites.

Variables	Post-transplant refractory ascites: Present (n = 14)	Post-transplant refractory ascites: Absent (n = 68)	P value
Fibrosis* (n, %)			
F0–F1	5 (36%)	53 (78%)	0.001
F2–F3	8 (57%)	15 (22%)	
F4	1 (7%)	0	
Activity* (n, %)			
A0–A1	8 (57%)	48 (71%)	0.32
A2–A3	6 (43%)	20 (29%)	
Steatosis† >30% (n, %)	0	8 (12%)	0.17
Sinusoidal distension (n, %)	6 (43%)	16 (23%)	0.13
Perisinusoidal fibrosis (n, %)	8 (57%)	14 (21%)	0.004
Centrolobular necrosis (n, %)	1 (7%)	8 (12%)	0.61

*According to METAVIR score.
†macrovesicular steatosis.

Table 3. Post-transplant characteristics in patients with and without refractory ascites.

Variables	Post-transplant refractory ascites: Present (n = 14)	Post-transplant refractory ascites: Absent (n = 68)	P value
Body mass index (kg/m^2)	26 ± 8	25 ± 5	0.42
Post-transplant diabetes (%)	43	19	0.04
Serum creatinine (μmol/l)	91 ± 27	96 ± 66	0.78
Tacrolimus (%)	100	95	0.7
Positive cryoglobulinemia (%)	79	22	0.005
SerumHCV-RNA ($\log_{10}$ UI/ml)*	6.4 ± 6.6	6.5 ± 6.7	0.9
HCV genotype			
Genotypes 1 and 4	71%	66%	0.7
Other genotypes	29%	34%	
Doppler ultrasound findings*			
Hepatic artery resistive index	0.67 ± 0.09	0.7 ± 0.16	0.4
Arterial systolic acceleration time (s)	0.05 ± 0.01	0.06 ± 0.01	0.12

*At the time of the onset of post-transplant ascites or at latest follow-up in those without ascites, in the absence of sustained virological response.

post-transplant diabetes was less frequent in those without ascites (15%) than in those with ascites (45%) with a difference at the limit of significance (P = 0.05). Similarly, perisinusoïdal fibrosis at 1 year was less frequent in those without ascites (23%) than in those with ascites (54%) with a difference at the limit of significance (P = 0.06).

Table 4. Variables associated with post-transplant refractory ascites: multivariate analysis.

Variable	Odd ratio	95% confidence interval	P value
Pretransplant refractory ascites	7.1	1.3–39	0.001
Fibrosis ≥stage 2 at 1 year	11.9	2.2–64	0.004
Presence of perisinusoidal fibrosis at 1 year	5.4	1.2–27	0.02
Positive cryoglobulinemia	7.3	1.3–41	0.02

Management of post-transplant refractory ascites and outcome

The initial management of refractory ascites was based on periodic paracentesis followed by infusion of albumin according to current recommendations in patients with cirrhosis [7,15,18].

Diuretics (furosemide and/or spironolactone) were stopped due to their inefficacy and their potential to deteriorate renal function. One patient who had early recurrence of refractory ascites concomitant to cryoglobulinemia-induced nephropathy (membranoproliferative glomerulonephritis) with renal insufficiency received antiviral therapy (combination of pegylated interferon and ribavirin) 4 months after transplantation for a total of 64 months. He had sustained virological response and ascites resolved thereafter. A total of four additional patients also received antiviral therapy and only one had sustained virological response. Longitudinal data concerning cryoglobulins were available in four patients with refractory ascites, who received antiviral therapy and who were positive for cryoglobulins before the initiation of therapy. Only one patient had sustained virological response and he remained cryoglobulin positive after the end of antiviral therapy. Among the three remaining patients without sustained virological response, cryoglobulin was still positive after discontinuation of antiviral therapy in two and became negative in one. In this patient, ascites persisted despite the absence of cryoglobulin.

Three patients had transjugular intrahepatic portosystemic shunt (TIPS) 22 to 37 months after transplantation. Ascites resolved in one of these three patients and persisted despite patent TIPS in 2. Three patients were listed for retransplantation among whom one was eventually retransplanted. As shown in Fig. 1, 5-year post-transplant survival was significantly lower in patients with refractory ascites compared to patients without refractory ascites (75% vs. 85%, P = 0.02).

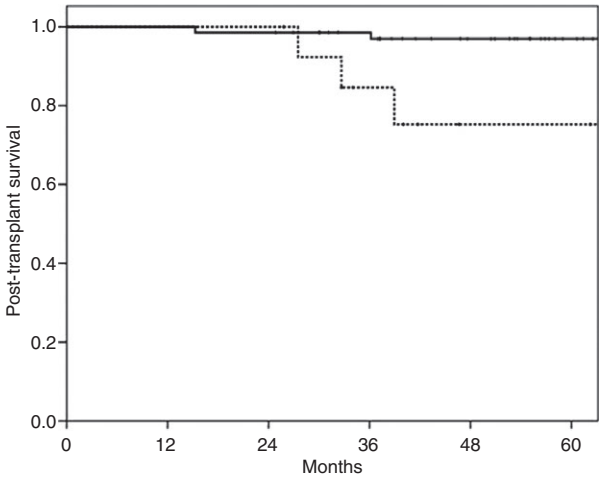

Figure 1 Post-transplant survival in HCV-infected patients with (dotted line) and without (continuous line) post-transplant ascites.

Discussion

Our data confirm the results of previous studies showing that post-transplant ascites in patients with recurrence of HCV infection most often occurs at an earlier stage of disease progression, before the stage of cirrhosis [6,8–10]. Even though in multivariate analysis, patients with a fibrosis score of 2 or more were more likely to develop ascites, only one of 14 patients with ascites had cirrhosis and 9 (64%) had F1–F2 fibrosis according to the METAVIR score [11]. None of the patients with refractory ascites had any evidence of hepatic outflow obstruction. In addition, among the nine patients who had transjugular liver biopsy with catheterization of the hepatic veins, hepatic venous pressure gradient was normal or moderately elevated, ranging from 5 to 17 mmHg. These values are in line with those observed after transplantation in HCV-infected patients with mild fibrosis but no ascites [19]. Therefore, a mild increase in HVPG can be observed in HCV-positive patients after transplantation, even in the absence of cirrhosis, but is not sufficient by itself to explain ascites. Indeed, markedly higher HVPG values are generally observed in nontransplanted cirrhotic patients with refractory ascites [20].

Interestingly, we found a statistically significant and independent relation between post-transplant cryoglobulinemia, a potential cause of vasculitis [21,22], and the occurrence of ascites. Patients positive for cryoglobulinemia post-transplantation were at more than seven times higher risk to develop ascites during the course of recurrent HCV infection compared to negative patients. Only a minority of patients who developed ascites (3 of 14) were found to be negative for cryoglobulinemia. These findings strongly suggest that cryoglobulinemia is a major predisposing factor for post-transplant ascites in HCV-infected patients. As we did not perform repeated determinations of cryoglobulinemia during post-transplant course, it is impossible to conclude definitely that cryoglobulinemia never had been transiently positive prior to ascites among those who were negative at the time ascites appeared. Due to the limited number of patients, it is also impossible to draw strength conclusions concerning the possible interactions between the course of cryoglobulinemia and the course of ascites. In one patient, cryoglobulinemia persisted after discontinuation of antiviral therapy despite SVR, a finding which has already been observed in nontransplanted patients [23].

We also found that the presence perisinusoidal fibrosis on 1-year post-transplant biopsy was a predisposing factor for refractory ascites, further supporting the concept that liver microangiopathy is involved in the occurrence of ascites. However, in this series, both cryoglobulinemia and perisinusoidal fibrosis were independently associated with ascites suggesting that other factors contribute to liver microangiopathy. Interestingly, diabetes which is another potential cause of microangiopathy [24] was significantly more frequent in patients with refractory ascites. Overall, 6 of 14 (42%) patients with refractory ascites had diabetes compared to 13/68 patients (20%) without ascites. Therefore, the potential role of diabetes, a common finding after liver transplantation, especially in HCV-infected patients [25] should be further explored in larger populations.

Mixed cryoglobulinemia is defined by circulating polyclonal immunoglobulins G, with or without rheumatoid factor, that precipitate when serum is cooled below the body temperature [26]. The production of cryoglobulin in HCV-infected patients may result from chronic immune stimulation of B cells. Large amounts of HCV-RNA can be isolated in the immune complexes [21]. Chronic hepatitis C is the main cause of mixed cryoglobulinemia but most HCV-infected patients with positive cryoglobulinemia remain asymptomatic. Only a minority (5–10%) develop overt vasculitis [26]. Vasculitis results from the deposition of circulating cryoglobulins and immunoglobulin M complexes containing HCV particles in vessels wall [27]. While cryoglobulinemia-associated vasculitis has been extensively studied in the skin, the kidney and the peripheral nervous system [28–30], little is known about the potential consequences of cryoglobulinemia on liver microcirculation and portal flow. One study has shown that among HCV-infected patients, there was a significant increase in sinusoidal T-cell lymphocytes in those positive for cryoglobulinemia compared to those negative for cryoglobulinemia which could result from ongoing antigenic stimulation [31]. In another series of HCV-infected patients, the presence of anti-endothelial cell auto antibodies was found to

be more frequent in those with cryoglobulinemia and/or patent vasculitis [32]. In the present study, neither sinusoidal T-cell lymphocytes nor anti-endothelial cell antibodies were investigated. However, as perisinusoidal fibrosis was more frequent in patients with post-transplant ascites, these findings suggest that independent of the extent of fibrosis intrahepatic microvascular changes related to cryoglobulinemia could be involved in the mechanisms of HCV-related ascites. However, more data are needed on the impact of cryoglobulinemia on liver microcirculation, especially in the context of liver transplantation. In addition, the reasons why ascites is not observed in nontransplanted HCV-infected patients with cryoglobulinemia and mild to moderate liver fibrosis should also be explored.

In this series, pretransplant refractory ascites was a predisposing factor for post-transplant ascites, even though post-transplant ascites occurred at an earlier stage of disease progression. No sufficient data were available to determine whether cryoglobulinemia also played a role in the occurrence of refractory ascites pretransplantation. However, it must be noted again that all the patients who had refractory ascites pretransplantation had cirrhosis with prominent portal hypertension. More specific studies are needed to clarify the interactions between pretransplant and post-transplant ascites.

This study is not without limitations. There was no serial determination of cryoglobulinemia at regular intervals. The number of events is relatively small and the odd ratios associated with each predictive factor should be interpreted with caution. No conclusion can be definitely drawn of the precise mechanisms of post-transplant ascites in HCV-infected patients. However, we found a strong correlation between cryoglobulinemia and post-transplant ascites which opens new perspectives in the field with potential therapeutic implications.

In conclusion, this study confirmed that refractory ascites may appear post-transplantation in HCV-infected patients without cirrhosis and in the absence of prominent portal hypertension. Post-transplant ascites in this population had a deleterious impact on the outcome. We found an independent correlation between both cryoglobulinemia and perisinusoidal fibrosis, and post-transplant ascites suggesting that liver microangiopathy related to cryoglobulinemia and possibly, other factors, could be involved in the mechanisms of ascites.

Authorship

ST: collected and analyzed data, wrote the paper. AA: collected data. CF and FD: designed study, analyzed data and wrote paper. VP: designed study and revised manuscript. HV and HB: collected data. JB and DV: revised manuscript.

Funding

The authors have declared no funding.

References

1. Berenguer M, Ferrell L, Watson J, *et al.* HCV-related fibrosis progression following liver transplantation: increase in recent years. *J Hepatol* 2000; **32**: 673.
2. Duvoux C, Firpi R, Grazi GL, Levy G, Renner E, Villamil F. Recurrent hepatitis C virus infection post liver transplantation: impact of choice of calcineurin inhibitor. *Transpl Int* 2013; **26**: 358.
3. Tanaka T, Selzner N, Therapondos G, Renner EL, Lilly LB. Virological response for recurrent hepatitis C improves long-term survival in liver transplant recipients. *Transpl Int* 2013; **26**: 42.
4. 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.
5. Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology* 2002; **122**: 889.
6. Lan BY, Landry GM, Tan VO, Bostrom A, Feng S. Ascites in hepatitis C liver transplant recipients frequently occurs in the absence of advanced fibrosis. *Am J Transplant* 2008; **8**: 366.
7. Salerno F, Guevara M, Bernardi M, *et al.* Refractory ascites: pathogenesis, definition and therapy of a severe complication in patients with cirrhosis. *Liver Int* 2010; **30**: 937.
8. Cirera I, Navasa M, Rimola A, *et al.* Ascites after liver transplantation. *Liver Transpl* 2000; **6**: 157.
9. Nishida S, Gaynor JJ, Nakamura N, *et al.* Refractory ascites after liver transplantation: an analysis of 1058 liver transplant patients at a single center. *Am J Transplant* 2006; **6**: 140.
10. Stewart CA, Wertheim J, Olthoff K, *et al.* Ascites after liver transplantation–a mystery. *Liver Transpl* 2004; **10**: 654.
11. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289.
12. Logge C, Vettorazzi E, Fischer L, Nashan B, Sterneck M. Cost-effectiveness analysis of antiviral treatment in liver transplant recipients with HCV infection. *Transpl Int* 2013; **26**: 527.
13. Fried MW, Shiffman ML, Reddy KR, *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975.
14. Tedeschi A, Barate C, Minola E, Morra E. Cryoglobulinemia. *Blood Rev* 2007; **21**: 183.
15. Moore KP, Wong F, Gines P, *et al.* The management of ascites in cirrhosis: report on the consensus conference

of the International Ascites Club. *Hepatology* 2003; **38**: 258.
16. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245.
17. Ko EY, Kim TK, Kim PN, Kim AY, Ha HK, Lee MG. Hepatic vein stenosis after living donor liver transplantation: evaluation with Doppler US. *Radiology* 2003; **229**: 806.
18. Gines P, Cardenas A, Arroyo V, Rodes J. Management of cirrhosis and ascites. *N Engl J Med* 2004; **350**: 1646.
19. Blasco A, Forns X, Carrion JA, *et al.* Hepatic venous pressure gradient identifies patients at risk of severe hepatitis C recurrence after liver transplantation. *Hepatology* 2006; **43**: 492.
20. Rossle M, Ochs A, Gulberg V, *et al.* A comparison of paracentesis and transjugular intrahepatic portosystemic shunting in patients with ascites. *N Engl J Med* 2000; **342**: 1701.
21. Sansonno D, Dammacco F. Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations. *Lancet Infect Dis* 2005; **5**: 227.
22. Terrier B, Krastinova E, Marie I, *et al.* Management of non-infectious mixed cryoglobulinemia vasculitis: data from 242 cases included in the CryoVas survey. *Blood* 2012; **119**: 5996.
23. Levine JW, Gota C, Fessler BJ, Calabrese LH, Cooper SM. Persistent cryoglobulinemic vasculitis following successful treatment of hepatitis C virus. *J Rheumatol* 2005; **32**: 1164.
24. Latry P, Bioulac-Sage P, Echinard E, *et al.* Perisinusoidal fibrosis and basement membrane-like material in the livers of diabetic patients. *Hum Pathol* 1987; **18**: 775.
25. Saliba F, Lakehal M, Pageaux GP, *et al.* Risk factors for new-onset diabetes mellitus following liver transplantation and impact of hepatitis C infection: an observational multicenter study. *Liver Transpl* 2007; **13**: 136.
26. Cacoub P, Saadoun D. Hepatitis C virus infection induced vasculitis. *Clin Rev Allergy Immunol* 2008; **35**: 30.
27. Jacobson IM, Cacoub P, Dal Maso L, Harrison SA, Younossi ZM. Manifestations of chronic hepatitis C virus infection beyond the liver. *Clin Gastroenterol Hepatol* 2010; **8**: 1017.
28. Daghestani L, Pomeroy C. Renal manifestations of hepatitis C infection. *Am J Med* 1999; **106**: 347.
29. Crowson AN, Mihm MC Jr, Magro CM. Cutaneous vasculitis: a review. *J Cutan Pathol* 2003; **30**: 161.
30. Caudai C, Maimone D, Almi P, *et al.* The potential role of hepatitis C virus in the pathogenesis of the neurological syndrome in chronic hepatitis C. *Gut* 1997; **41**: 411.
31. Carmack S, Taddei T, Robert ME, Mistry P, Jain D. Increased T-cell sinusoidal lymphocytosis in liver biopsies in patients with chronic hepatitis C and mixed cryoglobulinemia. *Am J Gastroenterol* 2008; **103**: 705.
32. Cacoub P, Ghillani P, Revelen R, *et al.* Anti-endothelial cell auto-antibodies in hepatitis C virus mixed cryoglobulinemia. *J Hepatol* 1999; **31**: 598.