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Immunological dysfunction during or after antiviral therapy for recurrent hepatitis C reduces graft survival

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Abstract

Introduction—Pegylated interferon and ribavirin (PEGIFN/RBV) therapy for recurrent hepatitis C after liver transplantation (LT) is associated with a lower sustained virological response (SVR) rate as well as more frequent side effects compared to non-transplant patients. We aimed to determine the incidence and clinical characteristics of LT recipients with recurrent hepatitis C who

developed immunological dysfunction (ID) during or after PEG-IFN/RBV therapy and to assess its impact on patient and graft survival.

Methods—Seventy-four deceased donor LT recipients with histological recurrence of hepatitis C were treated with PEG-IFN/RBV from 1/00 to 12/08. ID was defined as biopsy-proven rejection or moderate plasma cell hepatitis. Patients were followed up until death, re-LT or 30 September 2011.

Results—Twelve patients (16 %) had ID, 8 (10.7 %) had cholestasis without ID, while 54 had no ID/cholestasis during or after discontinuation of PEG-IFN/RBV therapy. Biopsy-proven acute cellular rejection prior to (hazard ratio = 4.87, $p = 0.009$) and type of immunosuppression at the time of initiation of PEG-IFN/RBV were the only independent predictors of ID. Patients who were on tacrolimus at the time of initiation of PEG-IFN/RBV had a significantly lower risk of ID compared to those who were on cyclosporine (HR 0.254, $p = 0.023$). Patients with ID had a trend toward a lower SVR rate (25 vs. 54 %, $p = 0.18$) and a significantly higher rate of graft failure (33 vs. 4 %, $p = 0.004$) compared to patients with no ID/cholestasis.

Conclusions—ID is common during or after PEG-IFN/RBV therapy for recurrent hepatitis C and frequently associated with decreased graft survival, trending toward low rates of SVR. Careful monitoring of liver biochemistries during or after PEG-IFN/RBV therapy with a low threshold to biopsy patients and particularly those receiving cyclosporine-based immunosuppression may improve outcomes in these patients.

Keywords

Liver transplantation; Recurrent hepatitis C; Immunological dysfunction

Introduction

The progression of recurrence of hepatitis C virus (HCV) infection after liver transplantation (LT), a universal phenomenon, occurs at an accelerated rate compared to non-transplant patients with the development of cirrhosis in 6–23 % of patients at a median of 3–4 years after LT [1–3]. As a result, many patients with recurrent HCV are treated with a combination of pegylated interferon and ribavirin (PEG-IFN/RBV) in an attempt to slow the rate of disease progression and to eradicate HCV-RNA.

Pre-emptive antiviral treatment in the first few weeks after LT to prevent HCV reinfection has been abandoned largely because of the poor tolerability, high rate of treatment discontinuation and low rate of sustained virological response (SVR) [4–6]. Most investigators have instead focused on the treatment of established recurrent HCV after the first 6 months of LT with stage 2 fibrosis [6, 7]. Nevertheless, SVR rates remain low, ranging from 25 to 48 % [7–10]. In addition, immunological dysfunction (ID), including acute cellular rejection (ACR), plasma cell/autoimmune hepatitis (AIH) and chronic rejection, is well described with IFN therapy for recurrent HCV [11–15].

Because of the uncertainty about the impact of ID and other histological abnormalities on clinical outcomes in LT recipients receiving PEG-IFN/RBV, we examined the incidence and

risk factors among LT recipients who developed ID during or after PEG-IFN/RBV therapy for recurrent HCV and its impact on graft survival.

Methods

Patient population and data collection

The present study included all adult (≥ 18 years) deceased donor liver transplant recipients with biopsy-proven recurrent hepatitis C who received a combination of PEG-IFN/RBV as initial treatment for recurrent HCV between 1 January 2000 and 31 December 2008. The present study excluded patients who had received living donor LT and those who had received standard or PEG-IFN monotherapy after LT for recurrent HCV. Patients were followed up until death, re-transplant or 30 September 2011. The study was approved by the Institutional Review Board.

Data on demographics, pre-transplant history of IFN-based treatment, donor age, cold ischemia time (hours), calcineurin inhibitor (CNI) type, mycophenolate use and duration of steroid therapy were recorded. Pre-treatment liver histology, HCV-RNA level, HCV genotype, complete blood count with platelets, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin and creatinine, duration of PEG-IFN/RBV, dose reductions and discontinuations, occurrence of side effects and use of growth factors were also recorded.

Pre- and post-treatment liver histology

Liver biopsies were performed prior to the beginning of PEG-IFN/RBV in all patients and were repeated in patients with worsening liver chemistries during or after PEG-IFN/RBV treatment to rule out rejection or other etiologies.

The decision to start PEG-IFN/RBV was made by the individual transplant hepatologist. In general, patients with established biopsy-proven recurrent HCV with ≥ 2 Ishak fibrosis score and no medical contraindications to antiviral treatment are recommended to start PEG-IFN/RBV treatment. Recurrent HCV was based on histological features of HCV without any evidence of rejection or biliary obstruction on pre-treatment liver biopsy. All the pre- and available post-treatment biopsies were re-reviewed using the classification of Fiel et al. by two pathologists (HA and BM) (Table 1). The diagnosis of ID was established based upon pathologist's consensus on overall diagnosis using Fiel's classification [12]. Patients were classified as follows on post-treatment biopsy: (0) other diagnosis if there were no histological features of HCV or AIH; (1) recurrent HCV without AIH features; (2) HCV with AIH features; (3) typical features of AIH [12]. ID was defined as presence or worsening of autoimmune features [based upon the overall impression of 2 (HCV with AIH features) or 3 (typical AIH)] or rejection during or after PEG-IFN/RBV therapy on post-treatment biopsy. The inflammation score was a composite score of portal inflammation (0–3), interface hepatitis (0–3), lobular hepatitis (0–3) and plasma cells (0–3) [12]. The severity of plasma cell infiltrate was based on the approximate ratio of plasma cells to the rest of the inflammatory infiltrate as described by Fiel et al. [12] (Table 1). Ishak score for fibrosis,

Nonalcoholic Steato-hepatitis Clinical Research Network score for steatosis and Banff classification for ACR and chronic ductopenic rejection were used to grade the biopsies.

Histological cholestasis, defined as canalicular (bilirubin in dilated canaliculae) or as periportal (periportal ductular proliferation, commonly accompanied by a few neutrophils), was graded as absent or present. If present, it was coded as mild (rare foci of periportal ductular proliferation and/or occasional bile filled canaliculae), moderate (peri-portal ductular proliferation involving half of the portal tracts) or severe (periportal ductular proliferation involving most of the portal tracts and/or numerous bile-filled canaliculae). Canalicular cholestasis never was more than occasional.

Treatment protocol

All patients received a combination of PEG-IFN (alpha-2a or alpha-2b)/RBV. The intended dose of PEG-IFN alpha-2a/alpha-2b were 180 mcg and 1.5 mg/kg body weight per week, respectively. The intended dose of RBV was 800–1,200 mg/day in two divided doses. The intended duration of therapy was 48 weeks regardless of HCV genotype. Blood chemistries were tested at week 0, every 2 weeks for the first 8 weeks, then every 4 weeks during treatment and at weeks 12 and 24 after treatment. HCV-RNA level was tested prior to treatment, every 12 weeks during treatment, at the end of treatment and 6 months post-treatment. Adverse events were reviewed by a transplant nurse every 2–4 weeks, and the patients were reassessed in clinic by their treating physician at least every 12 weeks.

Immunosuppression

Immunosuppression consisted of tacrolimus, mycopheno-late and prednisone. Cyclosporine was used in patients who were unable to tolerate tacrolimus. Prednisone was usually tapered off completely by 6 months to 1 year. Most patients were tapered to a single CNI by 1 year. The goal tacrolimus and cyclosporine levels after 3 months of LT at our institution were 6–8 and 110–150 ng/ml, respectively, during the study period. The CNI trough levels were expressed as times the upper limit of target range if they were higher and times the lower limit of target range if they were lower. The immunosuppression protocol remained the same during the entire study period.

Statistical analysis

Primary outcome was the occurrence of ID defined as presence or worsening of autoimmune features. The secondary outcomes were histological cholestasis, SVR and graft failure. Cholestasis was defined as presence, persistence or worsening of cholestasis in the absence of ID and without bile duct injury during or after PEG-IFN/RBV therapy on post-treatment biopsy. SVR was defined as undetectable HCV RNA 6 months after end of treatment. Graft failure was defined as death or re-LT.

Continuous and categorical variables were expressed as median (inter-quartile) and proportions, respectively. Kruskal-Wallis test and χ^2 , Fischer's exact or linear-by-linear association test were used to compare the continuous and categorical variables. Kaplan-Meier analysis was used to assess the cumulative probability of ID, patient/graft survival. Time to event was the initiation of PEG-IFN/RBV treatment to death, re-LT or end of

follow-up period. Cox regression and time-dependent Cox regression were used to assess predictors of ID and the impact of ID and histo-logical cholestasis on graft survival. Logistic regression analysis was used to assess the predictors of SVR. A p value of <0.05 was considered statistically significant.

Results

Description of cohort

Two hundred sixty-four patients received deceased donor LT for HCV during the study period. All but one of these patients had detectable HCV RNA after LT. Ninety patients were started on antiviral therapy, and 74 met the study criteria. Sixteen patients were excluded from the study because of the following reasons: treatment with either standard IFN or PEG-IFN monotherapy ($n = 15$) and recipient of living donor LT ($n = 1$) (Fig. 1). The baseline characteristics of the cohort are summarized in Table 2.

Pre-treatment laboratory and histologic features

The pre-treatment laboratory values are shown in Table 2. HCV genotype data were available for 61 patients of whom 44 (72 %) had genotype-1 infection. The median time between pre-treatment biopsy and PEG-IFN/RBV treatment was 11 weeks (5–22). The median pretreatment inflammation and fibrosis scores were 5 (4–7) and 2 (1–3), respectively. Only five patients (6.7 %) had 30 % plasma cells in the inflammatory infiltrate or plasma cells arranged in sheets or a cluster; none had centrilobular necrosis on the pre-treatment biopsy. Only two (2.7 %) had evidence of severe cholestasis but neither met the criteria for fibrosing cholestatic HCV.

Immunosuppression

Sixty patients (81 %) were on CNI monotherapy [tacrolimus, $N = 43$ (72 %); cyclosporine, $N = 17$ (28 %)], 13 (18 %) were on dual therapy (CNI + mycophenolate, $N = 8$; CNI + prednisone, $N = 5$), and one was on triple immunosuppression at the beginning of PEG-IFN/RBV. Eight of seventy-four patients were participating in the steroid avoidance protocol and received no steroid therapy from the time of LT. These eight patients were maintained on tacrolimus and mycophenolate for 6 months after the LT, followed by tacrolimus monotherapy.

Acute cellular rejection prior to PEG-IFN/RBV treatment

There were a total of 12 patients who had biopsy-proven ACR prior to PEG-IFN/RBV treatment after a median of 13.5 weeks (3–19) post LT. Six of them were treated with intravenous pulse steroids, one was treated with OKT3, two were treated with oral steroids, and three were treated by increasing the dose of CNI. Biopsy was repeated after completion of treatment, and all 12 had evidence of resolution of rejection.

PEG-IFN/RBV treatment

Figure 1 shows the development of the study cohort and outcomes of the 74 patients who met the study criteria and were treated with PEG-IFN/RBV. The median time from LT to

initiation of PEG-IFN/RBV was 72 weeks (41–126). Twenty-four (32 %) patients were started on treatment within the first year post-LT, 26 (35 %) during the second year, 20 (28 %) between years 2 and 5, and 4 (5 %) >5 years post-LT.

The median duration of PEG-IFN/RBV therapy was 46 weeks (18–51). An early virological response (EVR) with undetectable HCV-RNA at week 12 was seen in 37 patients (49 %). SVR was achieved in 33 patients (44.5 %) overall, 16 (36.4 %) of 44 patients with genotype-1, 11 (64.7 %) of 17 patients with non-1 genotypes, and 6 (46.2 %) of 13 patients with unknown HCV genotype. Non-1 genotype was significantly associated with SVR in the univariate analysis. However, EVR was the only independent predictor of SVR (odds ratio = 0.009 (95 % CI 0.001–0.11, $p < 0.0001$), after adjusting for baseline HCV-RNA, fibrosis score and genotype.

Thirty-five patients discontinued treatment before week 48, 23 early and 12 late. Thirteen and 10 patients discontinued treatment within 12 weeks and between 13 and 26 weeks of initiation of PEG-IFN/RBV, respectively. The reasons for treatment discontinuation in these 23 patients were ID ($n = 6$, including 1 with ACR), cholestasis ($n = 4$, one had SVR), acute myocardial infarction ($n = 3$), non-responder ($n = 3$), suicidal ideas ($n = 2$), thrombocytopenia ($n = 1$), severe myalgia ($n = 1$), flare of ulcerative colitis ($n = 1$), infection ($n = 1$) and severe anemia ($n = 1$). Twelve patients discontinued treatment between 27 and 45 weeks. Four of these 12 had end of treatment response, 5 were non-responders, 1 had cholestasis, and 1 had severe pancytopenia.

ID and histological cholestasis

ID and histological cholestasis were two distinct histological findings on post-treatment biopsies. None of the patients with histological cholestasis had ID. Table 3 shows the comparison of baseline characteristics of patients with ID [$n = 12$ (16 %)] or cholestasis [$n = 8$ (10.7 %)] based upon Fiel's classification on a post-treatment biopsy obtained during or after PEG-IFN/RBV therapy and those with no-ID/cholestasis [$n = 54$ (73.3 %)]. The median pre-treatment and end of treatment CNI levels in the groups with ID, histological cholestasis or no ID/cholestasis were similar (Table 3).

ID vs. no-ID/cholestasis—Baseline characteristics of the 12 patients with ID were similar to the 54 patients with no ID/cholestasis except for higher baseline serum AST ($p = 0.04$) and ALT ($p = 0.02$) levels and a lower percent receiving tacrolimus (25 vs. 81 %, $p = 0.002$) in the group with ID, but CNI trough levels at the initiation of PEG-IFN/RBV were similar (Table 3).

Laboratory findings at the time of ID diagnosis included a median AST of 167 IU/ml, ALT of 214 IU/ml, ALP of 147 IU/ml and total bilirubin of 0.9 mg/dl. The serum tacrolimus or cyclosporine trough level immediately prior to the biopsy that diagnosed ID was in the therapeutic range in 33 % of the patients in both groups. The biopsy of ID patients usually showed intense plasma-lymphocytic infiltrate in the portal triads with moderate interface hepatitis on the post-treatment biopsy. Inflammation included lymphocytes and plasma cells extending beyond the portal tract to surround periportal hepatocytes, a pattern that is typical of sporadic AIH. Antiviral therapy was discontinued in those patients who developed ID on

PEG-IFN/RBV. The tacrolimus dose was increased to achieve a trough level between 8 and 12 ng/ml. Oral steroids and mycophenolate/ azathioprine were also added to the regimen on a case-by-case basis. Those who were on cyclosporine were switched to tacrolimus.

The cumulative probability of ID was higher in patients who were on cyclosporine compared to those who were on tacrolimus (log-rank $p < 0.007$) (Fig. 2). The proportion of patients with ID were similar among patients treated with PEG-IFN alpha 2a or PEG-IFN alpha 2b.

The median duration of PEG-IFN/RBV therapy among these 12 patients was 29 weeks (12–55). Eight patients had ID after a median of 17 weeks (10–29) of PEG-IFN/RBV treatment. All eight had repeat biopsy because of an increase in liver enzymes and total bilirubin. One of these eight had ACR. PEG-IFN/RBV was stopped in all but one patient. The median duration of PEG-IFN/RBV therapy among four patients who developed late ID a median of 66 weeks (47–96) after discontinuation of PEG-IFN/RBV treatment was 57 weeks (50–59). Pre-treatment AST [hazard ratio (HR) = 1.003, $p = 0.041$], ALT (HR = 1.004, $p = 0.027$), and CNI type (tacrolimus vs. cyclosporine, HR = 0.102, $p = 0.001$), and acute rejection prior to PEG-IFN/RBV (acute rejection vs. no rejection, HR = 5.62, $p = 0.008$) were the significant predictors of ID on univariate analysis. CNI type prior to initiation of PEG-IFN/RBV (tacrolimus vs. cyclosporine, HR = 0.25; $p = 0.023$) and acute rejection prior to initiation of PEG-IFN/RBV (HR = 4.87, $p = 0.009$) were the only independent predictors of ID after adjusting for AST and ALT levels at the beginning of treatment. Patients who were on tacrolimus at the initiation of PEG-IFN/RBV had 75 % lower risk of developing ID compared to cyclosporine. There was a trend toward lower SVR in the ID group compared to the no ID/cholestasis group (27.3 vs. 54 %, $p = 0.18$).

Histological cholestasis vs. no ID/cholestasis—Eight patients (10.7 %) had new onset or worsening of histological cholestasis during or after discontinuation of PEG-IFN/RBV. Six of them developed cholestasis on PEG-IFN/RBV treatment [median 25.5 (11–48) weeks after start of treatment] and two developed cholestasis 16 and 34 weeks after discontinuation of PEG-IFN/RBV treatment. None of them had evidence of fibrosing cholestatic HCV on their pre-treatment biopsy.

Table 3 shows the baseline characteristics of patients with cholestasis and no ID/cholestasis. The biopsy showed proliferating bile ductules at the periphery of portal tracts, surrounded by edematous or fibrotic stroma, but with only scant inflammation. A significantly lower proportion of patients in the cholestasis group were on tacrolimus compared to the no ID/histological cholestasis group (50 vs. 82 %, $p = 0.02$). Similarly, 50 % of the patients with cholestasis were still on mycophenolate at the initiation of PEG-IFN/RBV therapy compared to 5.6 % patients with no ID/cholestasis ($p = 0.004$). The trough CNI levels were similar in both groups (Table 3). The donor age and cold ischemia time were similar in both groups (Table 3). Neither the type of immunosuppression ($p = 0.31$) nor the donor factors such as donor age ($p = 0.22$) and cold ischemia time ($p = 0.77$) predicted histological cholestasis.

An SVR was seen in two patients (25 %) in the cholestasis group compared to 28 (54 %) in the no ID/cholestasis group ($p = 0.25$).

ID vs. cholestasis group—There was no difference in the demographics, baseline laboratory values, pre-treatment histology or immunosuppression between the ID and cholestasis group. Graft failure rates as well as SVR rates were also similar in both groups.

Graft and patient survival

Ten (13.5 %) of the 74 treated patients experienced graft failure (death = 6; re-transplant = 4) after a median follow-up of 4.6 years after the initiation of PEG-IFN/RBV treatment. The 1-, 3- and 5-year patient and graft survivals for the entire cohort were 100, 96.6 and 89.1 % and 98.6, 93.9 and 83.9 %, respectively.

Graft failure was seen in four patients (33 %) in the ID group compared to only two patients (4 %) in the no ID group ($p = 0.004$). Four (50 %) patients in the cholestasis group and two (3.7 %) in the no ID/cholestasis group had graft failure ($p < 0.002$). One patient each in the ID group and cholestasis group had graft loss despite achieving SVR.

On multivariable analysis, development of ID or histo-logical cholestasis during or after treatment [HR = 6.43 (1.36–30.40), $p = 0.019$] and use of mycophenolate at the initiation of PEG-IFN/RBV therapy [HR = 6.52 (1.74–24.45), $p = 0.005$] were the only independent predictors of graft failure after adjusting for CNI type (tacrolimus vs. cyclosporine) and donor age.

Discussion

The present study demonstrated that ID during or after PEG-IFN/RBV therapy for recurrent HCV after LT was common and its occurrence increased the risk of graft failure. The novel finding of the present study was the association of acute rejection prior to initiation of PEGIFN/RBV treatment with ID. This risk was 4.8-fold higher among those who had acute rejection prior to initiation of PEG-IFN/RBV treatment compared to those who did not have acute rejection. Additionally, patients who were on tacrolimus had a lower risk of ID compared to those who were on cyclosporine at the initiation of PEG-IFN/RBV.

ID during or after the discontinuation of PEG-IFN/RBV therapy is a distinct clinical entity; however, it was under-reported in the literature until recently [9, 14, 16–20]. A recent multicenter case controlled study found the incidence of ID during or after discontinuation of PEG-IFN/ RBV therapy to be 7.2 % among HCV LT recipients managed at seven centers [19]. Although the incidence of ID was higher in our study compared to the previously reported average incidence in the recent multicenter study (16 % vs. 7.2 %) [19], the incidence across the center in that multicenter study varied from 3.2 % to 16.3 % [19]. Differentiating necro-inflammation due to recurrent hepatitis C, ACR or autoimmune hepatitis among LT patients with recurrent hepatitis C is challenging [21]. The present study used Fiel et al.'s externally validated scoring system to evaluate ID among LT recipients with recurrent hepatitis C [12, 19, 20].

Acute rejection is a multistep process and represents a cascade of events including alloantigen recognition, lymphocyte activation, clonal expansion and graft inflammation. PEG-IFN is an immunomodulator and may increase the risk of rejection in solid organ

transplant recipients. ID, manifested by intense necro-inflammation consisting of plasma lymphocytic cell infiltrate, is considered to be an extension of acute rejection. Based upon our data, we speculate that PEG-IFN-based therapy may trigger auto-immunity among those who already had an episode of acute rejection prior to initiation of antiviral therapy.

Our study identified that tacrolimus at the time of initiation of PEG-IFN/RBV was protective against the development of ID, despite a similar proportion of patients that received cyclosporine and tacrolimus having serum levels in the therapeutic target range prior to initiation of antiviral therapy. Cochrane review of randomized controlled trials of CNI showed the superiority of tacrolimus over cyclosporine in improving patient and graft survival and preventing acute rejection, including steroid-resistant rejection after LT [22]. In Fiel et al.s' [12] study, histological changes compatible with autoimmunity including AIH and rejection in post-transplant liver biopsy of LT recipients with HCV were more frequently observed in association with a recent lowering of maintenance immunosuppression (47 % of cases) or sub-therapeutic CNI levels (35 %). Although CNI levels were comparable at the initiation and at the end of PEG-IFN/RBV treatment in our study, it is plausible that patients on cyclosporine may need slightly higher target cyclosporine levels while on PEG-IFN/RBV treatment to suppress PEG-IFN mediated autoimmunity. However, this observation needs to be confirmed in a large prospective study.

We saw two distinct forms of ID in our study. Early ID was seen during or within 6 weeks of discontinuation of PEG-IFN/RBV therapy and often resulted in premature termination of PEG-IFN/RBV; it may reflect unmasking or exacerbation of autoimmune hepatitis by PEG-IFN, a phenomenon that is also seen in the non-transplant setting [23–27]. Late ID was seen 6 months after discontinuation of PEG-IFN/RBV therapy with no other precipitating cause. The late phenotype may represent a variant of denovo autoimmune hepatitis, which has been reported in 0.4–3.4 % of transplant recipients without previous auto-immune hepatitis [28, 29]. In general, management of ID during or after antiviral therapy consists of increasing the level of immunosuppression, which was done in ten patients in this series as well.

Female gender is usually associated with autoimmune disorders. Our data did not show an association between gender and ID. However, the proportion of females in the no ID/cholestasis group was slightly higher (35 %) compared to the ID (12 %) and cholestasis group (16 %). Although donor age did not predict ID or cholestasis, there was a trend toward higher donor age among those who developed ID and cholestasis compared to those with no ID/cholestasis.

Our study also identified a subset of patients who developed histological cholestasis (10.7 %) during or after discontinuation of PEG-IFN/RBV in the absence of biliary tract obstruction, which was associated with poor graft survival. None of these patients had evidence of fibrosing cholestatic hepatitis C in the pretreatment biopsy [30]. A large proportion of patients who developed cholestasis were on cyclosporine and on mycophenolate at the initiation of PEG-IFN/RBV therapy; however, none of these factors or the donor factors were significantly associated with cholestasis in the multivariable model. The question remains whether histological cholestasis is a harbinger of worse outcomes or

part of the histological spectrum of a more slowly evolving variant of fibrosing cholestatic HCV.

Like other studies, our study confirmed an overall SVR rate of 44.5 % and EVR as an independent predictor of SVR, after adjusting for genotype [8, 9, 31]. Patients who developed SVR had better graft survival [32]. Despite similar SVR rates in ID and non-ID group in our study, patients with ID demonstrated poor graft survival.

Limitations of our study include its retrospective design and small sample size. In addition, the lack of protocol biopsy may have underestimated the incidence of ID. Despite these limitations, we identified the new association of type of CNI and ID during or after discontinuation of PEG-IFN/RBV. Additionally, we also identified two distinct phenotypes associated with ID, which may represent two ends of the spectrum of autoimmunity with different pathophysiologic mechanisms.

In conclusion, our data demonstrate that the efficacy of PEG-IFN/RBV treatment for recurrent hepatitis C is challenged by adverse effects including ID and histological cholestasis, which are associated with reduced graft survival. Maintaining adequate levels of immunosuppression prior to and during treatment may prove worthwhile in decreasing the incidence of ID and histological cholestasis. However, this concept needs to be prospectively studied before any recommendations can be made.

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Abbreviations

AIH	Autoimmune hepatitis
HCV	Hepatitis C virus
ID	Immunological dysfunction
LT	Liver transplantation
PEG-IFN	Pegylated interferon
RBV	Ribavirin
SVR	Sustained virological response

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Fig. 1.

Outcome of patients treated with PEG-IFN/RBV therapy. *SVR* sustained virological response, *EOTR* end of treatment response, *Rx* treatment

Fig. 2. Unadjusted cumulative probability of ID stratified by type of immunosuppression (tacrolimus vs. cyclosporine)

Table 1

Grading of the biopsy (adapted from [12])

Overall assessment	Score
Other	0
HCV histological features without AIH features	1
HCV with AIH features	2
Typical of AIH	3
A. Portal inflammation	Score
Normal	0
Mild: sprinkling of inflammatory cells in occasional portal tracts	1
Moderate: dense inflammatory infiltrates in some portal tracts	2
Severe: dense inflammatory infiltrates in most portal tracts	3
B. Interface hepatitis	Score
None	0
Mild: focal, only seen in a few portal tracts	1
Moderate: affecting <50 % of the limiting plate in most portal tracts or continuous in few portal tracts	2
Severe: continuous in most portal tracts	3
C. Lobular activity	Score
None	0
Mild: scattered foci of parenchymal inflammation (<5 foci/10× field)	1
Moderate: numerous foci of parenchymal inflammation (>10 foci/10× field)	2
Severe: moderate as above plus bridging necrosis and/or collapse	3
D. Plasma cells	Score
Absent	0
Occasional (<5%)	1
Moderate (5-30 %)	2
Severe (>30 % or in sheets or clusters)	3
Total score (A, B, C and D)	0-12
Centrilobular necrosis	Score
Mixed inflammatory infiltrate (including plasma cells)	Yes/no
Fibrosis	0-6
Steatosis	0-3
Rejection activity index	0-9
Cholestatic changes	Yes/no
If yes	Mild/moderate/severe

Table 2

Baseline characteristics of the cohort

Variables	Median (inter-quartile range) or number (%)
Age at LT (years)	51 (48-53)
Caucasians	91.8 %
African-Americans	2.7 %
Others	2.7 %
Males	71 %
Donor age	42 years (24-49)
Genotype-1	53 (72 %)
Laboratory tests before the initiation of PEG-IFN/RBV therapy	
Log ₁₀ HCV RNA	6.39 IU/ml (5.69-6.85)
WBC	4.6/mm ³ (3.3-6.4)
Hemoglobin	14 g/dl (13-15)
Platelets	142,000/mm ³ (110,000-184,000)
Creatinine	1.1 mg/dl (0.9-1.2)
AST	151 IU/ml (83-215)
ALT	161 IU/ml (94-255)
ALP	153 IU/ml (103-200)
Total bilirubin	1.0 mg/dl (0.8-1.5)
Histology before the initiation of PEG-IFN/RBV therapy	
Inflammation score	5 (4-7)
Ishak fibrosis score	2 (1-3)
Plasma cells	
None	24 (32.4 %)
Occasional (<5 %)	31 (41.9 %)
5-30 %	15 (20.3 %)
>30 %	4 (5.4 %)
Cholestasis	
None	53 (71.6 %)
Mild	16 (21.6 %)
Moderate	3 (4.1 %)
Severe	2 (2.7 %)

Table 3

Clinical features of patients who developed immunological dysfunction, histological cholestasis or neither during or after PEG-IFN/RBV therapy

	ID (N = 12)	Histological cholestasis (N = 8)	No ID/histological cholestasis (N = 54)	p value
Age at LT (years)	51 (46-57)	50	51 (41-65)	0.62
Race/ethnicity				0.60
Caucasians	91.7 %	100%	92.6 %	
African-Americans	0%	0%	3.7 %	
Hispanics	8.3 %	0%	1.9 %	
Others	0%	0%	1.9 %	
Males	83.3 %	88 %	65 %	0.104
Donor age (years)	45 (30-54)	52 (33-57)	35 (23-46)	0.14
Cold ischemia time (h)	8.3 (6.8-9.4)	9.3 (7-9.7)	8.5 (7.3-10.1)	0.64
Genotype-1	7 (77.8 %)	5 (71.4 %)	31 (70.5 %)	0.83
Acute rejection	5 (42 %)	2 (25 %)	5 (9.3 %)	0.038
Laboratory tests before the initiation of PEG-IFN/RBV therapy				
Log HCV RNA (IU/ml)	6.47 (5.93-7.02)	6.35 (6.42-6.90)	6.25 (5.69-6.83)	0.23
WBC (/mm ³)		5.75 (3.9-6.5)	4.5 (3.3-4.6)	0.29
Hemoglobin (g/dl)	5.6 (3.5-7.7)	13.6 (13.4-14.2)	14.1 (13-14.9)	0.26
Platelets (1,000/mm ³)	14.8 (13.1-16.7)	140 (115-152)	143 (112-184)	0.77
Creatinine (mg/dl)	138 (110-195)	1.1 (1.0-1.2)	1.0 (0.9-1.2)	0.35
AST (IU/l)	1.1 (1.0-1.2)	186 (127-288)	134 (76-177)	0.041
ALT (IU/l)	167 (134-287)	173 (98-288)	135 (71-219)	0.047
ALP (IU/l)	214 (175-124)	173 (115-238)	144 (101-180)	0.59
Total bilirubin (mg/dl)	146 (105-350)	1.15 (1.0-3.3)	1.0 (0.8-1.4)	0.45
	0.9 (0.5-1.7)			
Histology before the initiation of PEG-IFN/RBV therapy				
Inflammation score	5.5 (4-7)	6.5 (4-7)	5 (4-7)	0.17
Portal inflammation	2 (2-3)	2 (1-2)	2 (1-3)	
Interface hepatitis	1 (1-2)	1 (1-2)	1 (1-2)	
Lobular hepatitis	1 (1-2)	1 (1-2)	1 (1-2)	
Plasma cells				0.06
None	2 (16.7 %)	2 (25 %)	20 (37 %)	
Occasional (<5 %)	6 (50 %)	2 (25 %)	23 (42.6 %)	
5-30 %	3 (25 %)	3 (37.5 %)	9 (17 %)	
>30 %	1 (8.3 %)	1 (12.5 %)	2 (3.7 %)	
Fibrosis score	3 (2-4)	3 (2-4)	2 (1-3)	0.57
Cholestasis				0.27
None	6 (50 %)	5 (62.5 %)	42 (77.8 %)	
Mild	5 (41.7 %)	2 (25 %)	9 (16.7 %)	

	ID (N = 12)	Histological cholestasis (N = 8)	No ID/histological cholestasis (N = 54)	p value
Moderate	1 (8.3 %)	1 (12.5 %)	1 (1.9 %)	
Severe	0 (0 %)	0 (0 %)	2 (3.7 %)	
Immunosuppression				
CNI levels pre-treatment (XULN)	1.0	0.97	1.0	0.39
CNI levels at EOT (XULN)	0.75	0.73	0.8	0.33
Tacrolimus/cyclosporine	3 (25 %)/9 (75 %)	4 (50 %)/4 (50 %)	44 (81 %)/10 (19 %)	0.002
Mycophenolate	2 (16.7 %)	4 (50 %)	3 (5.6 %)	0.001
Steroid duration after LT (months)	7 (2.8-10.5)	3.5 (2.5-12)	4.8 (2-9)	0.63
PEG-IFN/RBV treatment duration (weeks)	29 (13-55)	24 (9-42)	47 (31-51)	0.26
Time from LT to treatment (weeks)	64 (42-108)	110 (57-163)	72 (41-118)	0.56

Kruskal-Wallis test used for the continuous variables and χ^2 , Fisher exact and linear-by linear association test used for categorical variables