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High Incidence Of Allograft Dysfunction In Liver Transplant Patients Treated With Peg-Interferon Alfa-2b And Ribavirin For Hepatitis C Recurrence: Possible De Novo Autoimmune Hepatitis?

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Key words: Liver Transplantation, HCV recurrence, Peg-Interferon, De Novo Autoimmune Hepatitis, Rejection.

Abbreviations:

- AIH: autoimmune hepatitis
- LT: liver transplant
- IFN: interferon
- PEG-IFN: pegylated-interferon
- ALT: alanine aminotrasferase
- G-CSF: Granulocyte colony-stimulating factor
- EPO: Erythropoietin
- ANA: antinuclear antibodies
- ASMA: anti-smooth-muscle antibodies
- SVR: sustained virological response
- pANCA: antineutrophil cytoplasmic antibodies
- SLE: Systemic Lupus Erythematosus
- AMA: anti-mitochondrial antibodies

ABSTRACT

BACKGROUND/AIMS Interferon may trigger autoimmune disorders in immunocompetent patients, including Autoimmune Hepatitis. To date, no cases are described in liver transplanted patients.

METHODS 9/44 liver transplanted patients receiving Peg-Interferon alpha-2b and Ribavirin for at least six months for HCV recurrence, developed graft dysfunction in spite of on-treatment HCV-RNA clearance in all but one cases. Laboratory, microbiological, imaging and histological evaluations were performed to identify the origin of graft dysfunction. International Autoimmune Hepatitis scoring system was also applied.

RESULTS In all cases, infections, anastomosis complications and rejection were excluded, while the Autoimmune Hepatitis score suggested a "Probable Autoimmune Hepatitis" (score from +10 to +14). Three patients developed other definite autoimmune disorders (overlap AMA-positive Cholangitis, Autoimmune Thyroiditis and Systemic Lupus Erythematosus, respectively). In all cases, pre-existing Autoimmune Hepatitis was excluded. Anti-lymphocyte antibodies in immunosuppressive induction therapy resulted associated with the development of the disorder, while the use of G-CSF to treat interferon-induced neutropenia showed a protective role. Antiviral treatment withdrawn and prednisone treatment resulted in different outcomes (5 remissions, 4 graft failure with 2 deaths).

CONCLUSIONS *De Novo* Autoimmune Hepatitis should be considered in differential diagnosis along with rejection in liver transplanted patients developing graft dysfunction while on interferon treatment.

BACKGROUND AND AIMS

De novo autoimmune hepatitis (AIH) has been recently recognized as a new type of graft dysfunction affecting liver transplant (LT) recipients without a previous history of AIH. [1][2]. Diagnosis requires exclusion of alternative causes of allograft dysfunction and a clinical phenotype resembling classic AIH. Since the classic phenotype may be modified by immunosuppression, the International Autoimmune Hepatitis Group scoring system [3] may be inappropriate for diagnosis, that may consequently depend more heavily on the exclusion of other causes of allograft dysfunction. Responsiveness to corticosteroid remains an important diagnostic cornerstone. Hypotheses for AIH involve trigger factors, such as viruses or toxins [4][5], and a genetic predisposition. [6][7] The loss of self-tolerance may be due to impaired negative selection of autoreactive immunocytes [8], virus-induced polyclonal activation of lymphocytes cross-reactive to self-antigens (molecular mimicry) [9][10] and uncovering of cryptic autoantigens from inflammatory-damaged tissues. [11]

In the setting of LT, calcineurin inhibitors may impair the thymic negative selection of autoreactive cells [12] and prevent the apoptosis of autoreactive lymphocytes [13], enhancing autoreactivity. Hepatotropic viruses, whose incidence is greater because of immunosuppression, may lead to enhancement of MHC expression and lymphocytes polyclonal stimulation, generating, through the mechanism of molecular mimicry [14], an autoreactive, self-perpetuated immune response. It is known that interferon (IFN), because of its immunomodulatory effects, may trigger autoimmune disorders, including AIH [15][16], in immunocompetent patients, but there are no reports on the development of AIH in LT recipients receiving IFN. We report a form of graft dysfunction with features of de novo AIH that occurred in LT patients receiving pegylated-IFN (PEG-IFN) for hepatitis C recurrence.

PATIENTS AND METHODS

From October 2001 to April 2004, 54 consecutive LT recipients with recurrent hepatitis C were enrolled in a study protocol on antiviral treatment with PEG-IFN alfa-2b (Peg-Intron®, Schering-Plough) 1.0 µg/Kg/week and Ribavirin (Rebetol®, Schering-Plough) 800-1200 mg/day for at least 6 months.

Inclusion criteria were: LT for HCV related cirrhosis; elevated (> 1.5 x upper normal value) alanine aminotransferase (ALT); detectable serum HCV-RNA by qualitative assay (HCV TMA, Bayer Diagnostics); histological features compatible with HCV reinfection.

Patients aged <18 years, or with decompensated liver disease, HBV or HIV infection, haemoglobin <10 g/dL, white blood cell count <1.500/µL, platelet count <50.000/µL, endogenous creatinine clearance <50 ml/min, cardiovascular and psychiatric disease, ongoing alcohol abuse, histological evidence of rejection and previous post-LT treatment with PEG-IFN were excluded. A previous post-LT antiviral treatment with standard IFN was not considered an exclusion criterion.

Liver biopsy was performed before starting therapy and evaluated by an experienced pathologist. Diagnosis of recurrent hepatitis was based on the presence of portal, periportal and lobular inflammation, with lobular acidophilic bodies and/or lobular hepatocytolysis. Histological activity index was assessed according to the Knodell scoring system [17]. The Banff scoring system [18] was applied to exclude acute cellular rejection. Features of chronic rejection (loss of interlobular bile ducts in ≥50% of portal tracts accompanied by arteriopathy affecting hepatic artery branches at the hilum) [19] were also excluded.

All patients gave written informed consent to participate in the study that was conducted according to the principles of the Helsinki declaration.

Efficacy and safety were assessed by clinical and laboratory evaluations at each visit (performed monthly until 6 months after the cessation of treatment). HCV-RNA viral load in serum was determined by quantitative assay (third-generation HCV bDNA 3.0, Bayer Diagnostics) before starting therapy, at the end of the first month and every 3 months thereafter until the end of follow-

up; a qualitative assay was used when HCV-RNA fell below its detection limit. Non-organ specific autoantibodies were tested before therapy and every 3 months thereafter, by indirect immunofluorescence. [20]

Granulocyte colony-stimulating factor (G-CSF) (Granulokine®, Roche, Italy) and Erythropoietin (EPO) (NeoRecormon®, Roche, Italy) were used in cases of neutrophil count and haemoglobin falling below 1,000/ μ L and 10 g/dL, respectively. Treatment was stopped in the event of severe side-effects and/or sustained cytopenia despite G-CSF and EPO administration.

During the study period, 9 patients developed an unexpected form of graft dysfunction in spite of on-treatment virological response (qualitative HCV-RNA negative) in 8 of them.

According to protocol, they underwent laboratory tests evaluating calcineurin-inhibitor serum levels, non-organ specific autoantibodies, qualitative HCV-RNA and microbiological tests. Tests were also performed to verify regular patency of vessels and biliary tract. Liver biopsy was repeated, and if acute or chronic rejection and HCV reactivation were excluded, morphological criteria of the International Autoimmune Hepatitis Group [3] (interface hepatitis, plasma cells in the inflammatory infiltrate, rosetting of periportal hepatocytes and biliary changes) were assessed. HLA typing from donor and recipient was utilized to calculate the AIH score.[3] The results of the typing were compared and for each locus the number of mismatches was scored as 0, 1 or 2 on the basis of the number of donor's specificities not shared with the recipient for a total amount of mismatches variable from 0 to 6.

STATISTICAL ANALYSIS

Data were analysed using non-parametric tests, including the Mann-Whitney U test and Fisher's exact test. A p value less than 0.05 was considered to be statistically significant. All data analyses were conducted using the Statistical Package for Social Science (SPSS Version 11.5).

RESULTS

54 consecutive LT recipients with recurrent hepatitis C were enrolled in the study (M/F 36/18. Mean age 57 years, range 22-67. Median time after LT 17.5 months, range 1-151. Genotype 1 and 4 46 patients, genotype 2 and 3 8 patients). Induction immunosuppression consisted of thymoglobulin in 5 patients (9%), steroids in 46 (85%), other in 3 (6%).

Maintenance immunosuppression consisted of cyclosporine or tacrolimus (with also steroids in 14 patients), maintained in therapeutic range in relation to the time of LT. The five patients who received induction therapy with thymoglobulin were maintained at lower tacrolimus serum level in order to achieve tolerance.[21]

Ten patients stopped therapy before the expected 6 months because of liver decompensation (n=4), intolerance to treatment (n=4), liver abscess (n=1) and de novo hepatitis B (n=1).

Among the 44 patients treated for at least 6 months, 9 (17%) presented unexpected liver function test abnormalities in spite of on-treatment virological response in 8 of them. They were evaluated according to the protocol and in all cases infections, anastomoses complications, and acute or chronic rejection were excluded, while the application of the AIH score suggested the diagnosis of "probable AIH". Reevaluation of baseline data excluded the diagnosis before therapy in all cases. In all patients, antiviral treatment was withdrawn, and a course of prednisone (1 mg/Kg) was started. A brief description of such cases follows (Table 1).

Table 1. Main clinical and laboratory features of patients who developed graft dysfunction

					Before antiviral treatment				During development of graft dysfunction							
Patient	Sex	Age	Months After LT	HCV Genotype	Knodell score	Autoantibodies	Ig *	AIH score	HVC RNA by PCR	Knodell score	Autoantibodies	Ig *	AIH score	Other findings	SVR	Outcome
1	M	65	6	1	4-3-4-3	Negative	1.5	+2	Negative	5-3-4-3	ANA 1/40 ASMA 1/40	2	+14	No	n.a.	Death for variceal bleeding
2	F	61	62	1	4-3-3-1	ANA 1/40	0.9	+5	Negative	4-3-4-3	ANA 1/40	1.7	+12	Macrovesicular Steatosis 40%	n.a.	Death for graft failure
3	F	62	7	4	3-1-3-1	Negative	0.5	+1	Negative	4-1-3-1	ANA 1/40	1	+10	Macrovesicular Steatosis 40%	Yes	Remission with steroids
4	M	59	25	1	3-1-3-1	Negative	1.2	+5	Negative	5-1-4-1	ANA 1/320	1.5	+10	Ductopenia ¹	No	Improvement with steroids despite HCV relapse
5	M	58	35	1	3-1-3-1	Negative	0.7	-1	Negative	4-1-3-3	ANA 1/40	0.9	+14	Ductopenia ¹ Autoimmune thyroiditis	Yes	Remission with triple immunosuppression
6	M	60	12	1	1-3-1-1	ANA 1/40	0.8	+3	Positive	3-3-3-1	anti-dsDNA 1/10.240	1.9	+10	Macrovesicular Steatosis 60%	No	Improvement with steroids despite HCV relapse
7	M	55	19	1	4-3-3-1	ANA 1/640 ASMA 1/160	1.2	+5	Negative	4-3-3-3	c-ANCA 1/80 anti-dsDNA 1/80	1.9	+11	Cholangitis SLE	No	Improvement with steroids despite HCV relapse
8	F	60	3	2	3-1-3-1	Negative	1.1	+1	Negative	4-3-4-1	ASMA 1/40	1.5	+10	Cholangitis Hepatic artery stenosis ² Bile duct stenosis ²	Yes	Reenlisted for LT
9	M	66	1	1	3-1-3-1	Negative	1.2	0	Negative	5-1-4-3	AMA 1/40	2.2	+10	AMA positive cholangitis ³	Yes	Graft failure

Knodell score: periportal necrosis - intralobular degeneration and focal necrosis - portal inflammation - fibrosis

*Ig: immunoglobulin level (x upper normal range)

SVR: sustained virological response

n.a.: not available (negative at time of death)

1. Ductopenia did not fulfil criteria for chronic rejection

2. These complications occurred two months after the diagnosis of de novo AIH

3. Diagnosis of AIH/primary biliary cirrhosis overlap syndrome

Patient 1 started antiviral treatment 6 months after LT for a severe reinfection with advanced fibrosis. HCV-RNA tested negative by PCR after the first month of treatment. At month 6, an ALT flare occurred, and anti-nuclear antibodies (ANA) (titre 1/40) and anti-smooth-muscle antibodies (ASMA) (titre 1/40) appeared. Liver biopsy showed severe interface hepatitis with plasma cells infiltration and rosettes. The AIH score was 14. Antiviral treatment withdrawn and steroid therapy lead to a rapid biochemical response. Nevertheless, he died one month later because of variceal bleeding. At that time, qualitative serum HCV-RNA was still negative.

Patient 2 started treatment 62 months after LT. ANA was positive (1/40) at baseline. Serum HCV-RNA tested negative from month 3. In spite of persistent HCV-RNA negativity, at month 6 she presented jaundice and an ALT flare, with histological evidence of severe interface hepatitis, mild plasmacellular infiltration, rosettes, macrovesicular steatosis and worsening of fibrosis compared to baseline. The AIH score was 12. Antiviral treatment was stopped and corticosteroids were given without a significant improvement in liver tests. The immunosuppressive regime was therefore switched from cyclosporine to tacrolimus plus sirolimus. Nevertheless, she presented a relentless course of cholestatic hepatitis with progressive liver failure and died 3 months later. HCV-RNA by PCR was still negative.

Patient 3 started treatment at month 7 after LT, achieving rapid viral clearance. At month 10, an ALT flare occurred and ANA appeared (1/40), with histological evidence of interface hepatitis without plasmacellular infiltration, rosettes and macrovesicular steatosis. The AIH score was 10. Antiviral treatment withdrawn and steroids therapy lead to normalization of liver tests. Nine months later, she is still receiving low doses of steroid, with normal ALT and sustained virological response (SVR).

Patient 4 started treatment 25 months after LT. The virological response was achieved at month 6, when ANA became positive at high titre (1/320). At the end of treatment (month 12) an ALT flare was seen and liver biopsy showed interface hepatitis, plasmacellular infiltration and rosettes. Mild ductopenia was also present, but criteria for chronic rejection were not fulfilled. The AIH score was 10, and steroids were started with ALT normalization, despite a virological relapse 2 months later. He is still receiving low doses of steroids and is in good condition with normal ALT values.

Patient 5 started treatment 35 months after LT, obtaining a virological response after 3 months. At month 6, he developed jaundice with an ALT flare. ANA became positive at low titre (1/40) and liver biopsy showed interface hepatitis, plasmacellular infiltration and rosettes. Mild ductopenia was also present, without fulfilling criteria for chronic rejection. Autoimmune thyroiditis was also diagnosed. The AIH score was 14. Antiviral treatment was stopped, and steroid treatment was started. No improvement in liver tests was seen and serum bilirubin further increased. Liver biopsy was repeated one month later confirming the previous picture. Immunosuppressive regime was switched from cyclosporine and steroids to a combined treatment of tacrolimus, sirolimus and steroids with normalization of liver tests and maintenance of SVR.

Patient 6 started treatment one year after LT, clearing virus at month 6. One month later a mild increase in ALT level occurred, together with high titre of anti-dsDNA (1/10.240) (ANA 1/40 was present at baseline) and HCV-RNA reappearance. Liver histology showed interface hepatitis, plasmacellular infiltration, rosettes and macrovesicular steatosis. The AIH score was 10. Antiviral treatment withdrawn and steroid therapy allowed ALT normalization.

Patient 7 started treatment 19 months after LT, achieving virus clearance 6 months later. At baseline, ANA (1/640 homogeneous pattern) and ASMA (1/160) were positive. At month 11 he

developed polyserositis and migrant arthritis. Perinuclear staining for antineutrophil cytoplasmic antibodies (pANCA) and anti-dsDNA (1/80) became positive. According to ARA criteria [22], the diagnosis of Systemic Lupus Erythematosus (SLE) was made. Low dose steroids led to remission of SLE, and antiviral treatment was stopped at the end of month 12, achieving an end-of-treatment virological response. Two months later, although HCV-RNA was still negative, serum bilirubin and ALT increased. Liver biopsy showed interface hepatitis, rosettes, cholangitis, ductular proliferation and biliary regression. The AIH score was 11. The steroid dose was increased to 1 mg/Kg, with normalization of liver tests 1 month later although HCV relapse.

Patient 8 started treatment 3 months after LT, clearing virus from the first month of treatment. At month 12, an increase of ALT, alkaline phosphatase and bilirubin was observed. No vessel or biliary tract abnormalities were detected. ASMA at low titre (1/40) became positive and liver histology showed interface hepatitis, rosettes and cholangitis. The AIH score was 10. Antiviral treatment was stopped, and steroids were started with an improvement in liver tests. However, two months later, an increase in liver tests occurred again and right hepatic artery stenosis was diagnosed, with multiple right segmentary bile duct stenosis. Bilioplasty was unsuccessful and she was reenlisted for LT because of a progressive worsening of liver function.

Patient 9 started treatment 1 month after living donor LT. No response was achieved and treatment was stopped at month 12. One month later, he developed jaundice and an ALT flare. No abnormalities of extrahepatic bile ducts were found. HCV-RNA tested negative and anti-mitochondrial antibodies (AMA) (1/40) appeared (negative in the donor). Liver biopsy showed biliary aggression, ductular proliferation, severe interface hepatitis and abundant rosettes. Despite the cholestatic features, the AIH score was 10. The diagnosis of AIH and Primary Biliary Cirrhosis overlap syndrome was thus made. The patient was unsuccessfully treated with steroids and high doses of ursodeoxycholic acid. Six months later, HCV-RNA persisted negative but liver function progressively deteriorated.

In order to identify potential risk factors related to the development of graft dysfunction suggestive for de novo AIH, several variables were analysed. The use of anti-lymphocyte antibodies as induction therapy after LT proved to be significantly associated with the development of de novo AIH ($p=0.03$), while the use of G-CSF to treat neutropenia during PEG-IFN treatment showed a protective role ($p=0.02$). In particular, among the patients who developed de novo AIH, 3 received anti-lymphocyte antibodies, and none received G-CSF. No association was found between development of de novo AIH and either the recipient's or the donor's HLA-DR3/DR4, or HLA mismatches. The finding of non-organ specific autoantibodies at baseline did not seem to increase the likelihood of graft dysfunction.

In the entire population of 54 treated patients, in addition to the 9 cases reported above, the following uncommon adverse events were observed: autoimmune gastritis in 2 cases, and late hepatic artery stenosis in 3 further cases in addition to patient 8 in this series.

DISCUSSION

De novo AIH after LT is a newly recognised condition affecting patients transplanted for disorders other than AIH.[1][2] Risk factors and pathogenesis for this disorder remain unknown, and minimum criteria for diagnosis have not been standardised. [23]

To the best of our knowledge, no cases of de novo AIH have been described in LT patients receiving IFN or PEG-IFN for recurrent hepatitis C, although it is known that in immunocompetent patients IFN may trigger autoimmune disorders, including AIH.[16]

In this study, we describe the occurrence of a peculiar form of graft dysfunction in LT recipients receiving PEG-IFN alfa-2b and Ribavirin for hepatitis C recurrence. During or shortly after the

treatment, 9/54 patients (17%) experienced unexplained abnormalities of liver tests in spite of HCV-RNA clearance in most cases (8/9). Graft rejection, anastomosis complications, and concomitant infections were excluded in all cases. The laboratory and histological characteristics (autoantibody appearance or titre increase, severe interface hepatitis with plasmacellular infiltration and rosettes) of these cases led us to hypothesise the occurrence of de novo AIH. By applying the AIH scoring system [3], in all patients we obtained a score suggesting “probable AIH”. Interestingly, before treatment, the AIH score was clearly negative in all cases. The effects of immunosuppression, concomitant viral infection and drug toxicity make the diagnosis of AIH in LT patients with hepatitis C recurrence particularly difficult. In fact, these confounding factors can influence the intensity of plasmacellular infiltration in the liver, the serum levels of γ -globulins and the titre of autoantibodies. Considering these limitations, although not validated in this context, a positive AIH score [3] in this setting could be more meaningful than in non-transplanted patients. The fact that 3 out of 9 patients in this series also showed other definite autoimmune disorders, such as thyroiditis, overlap syndrome and SLE, reinforced the hypothesis of autoimmune-mediated graft dysfunction. Furthermore, other autoimmune manifestations occurred in the entire study population, such as autoimmune gastritis (n=2) and late hepatic artery stenosis (n=4) without other hypothetical risk factors other than PEG-IFN treatment, as we previously described. [24]

As compared to the reported value of about 4% in untreated patients [1][2], the high incidence of de novo AIH in our series suggests a potential pathogenetic role of the antiviral treatment. We believe that, in a clinical setting characterised by several conditions favouring autoimmune reactions, PEG-IFN, by virtue of its immunomodulatory effects, may trigger autoimmune disorders. The fact that all but one case occurred once the patients became HCV-RNA negative, may suggest that a vigorous immune response promoting virus clearance may favour tissue damage and consequent cryptic antigen release in a context of MHC interferon-induced up-regulation. Epitope spreading theory, defined as diversification of epitope specificity from the initial focused to cryptic epitopes on other proteins [reviewed in 25], supports the hypothesis that tissue damage during an immune response can lead to the priming of reactive lymphocytes, regardless of the specificity of the initial insult.

When we analysed possible risk factors related to development of de novo AIH, we did not find an association either with HLA aplotype, as reported by other authors [2], or with the finding of non-organ specific autoantibodies at baseline. Interestingly, the use of thymoglobulin was significantly associated with the occurrence of de novo AIH, while the use of G-CSF seems to have a protective role. We do not have a definitive explanation for these observations, but it is well known that thymoglobulin has relevant and long-term effects on cellular response. In fact, treated patients show a persistent low percentage of T cells for at least 2 years post-treatment, with low CD4/CD8 ratios and drops in CD25+ T cells.[26] We can argue that the relative increase in CD8 with loss of the inhibitory role of CD25+ cells, combined with the lower serum tacrolimus levels maintained in these patients in order to favour tolerance, can be permissive for development of Th1-mediated autoimmune disease.

The protective effect of G-CSF may derive from its known immune modulator effects on human CD4+T cells, skewing T-cell differentiation toward the Th2 phenotype, with consequent suppression of T cell alloreactivity.[27]

When patients developed de novo AIH, antiviral treatment was stopped and steroids were started with different outcomes: two patients died within 3 months because of variceal bleeding and liver failure, both being HCV-RNA negative; two other patients developed progressive liver failure, maintaining SVR; the remaining five are alive in good condition on maintenance steroid therapy, with 2 SVR.

In conclusion our findings suggest the occurrence of a new type of graft dysfunction in LT patients receiving PEG-IFN plus Ribavirin, not related to rejection and with a features compatible with de novo AIH. We are aware that autoimmunity by definition is characterized as a loss of tolerance

towards self-antigens, so the use of this term to define hepatitis affecting an allogenic organ is questionable. However until better knowledge of the true pathogenesis of this entity is acquired, the overall clinical, histological and immunological characteristics of these disorders strongly suggest an autoimmune mechanism, and de novo AIH should be considered in the differential diagnosis along with rejection.

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All authors declare no competing interests.

Prof. P. Andreone

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