Acute liver graft cellular rejection after interferon-free antiviral treatment for HCV infection. Is there a risk? A warning about three cases"

G. Dahlqvist[1], M. Komuta[2], L. Coubeau[3], Y. Horsmans[1]

(1) Hepatogastroenterology Department Cliniques Universitaires Saint-Luc, Brussels, Belgium ; (2) Anatomopathology Department Cliniques Universitaires Saint-Luc, Brussels, Belgium ; (3) Abdominal Surgery Department Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Abstract

All patients transplanted for hepatitis C (HCV)- related cirrhosis will experience a recurrence of the viral disease on the liver graft with an accelerated course of the disease and a progression to advanced liver fibrosis in up to 50% of the patients at 5 years post-liver transplantation. HCV infection is a high risk for graft lost.

We report here three cases of patients transplanted for hepatocellular carcinoma on HCV-related cirrhosis. All cases experienced an acute cellular rejection after the end of HCV therapy with direct acting antivirals (DAAs).

We thus advocate for a close monitoring of tacrolimus and liver tests even a few months after the end of the treatment. Clinicians using DAAs after liver transplantation should be aware of the dynamics of tacrolimus levels during therapy and immunological changes that can occur even several weeks (or months) after the end of DAA treatment. (Acta gastroenterol. belg., **2019**, 82, **53-56**).

Keywords : Liver transplantation, hepatitis C, direct acting antivirals.

Introduction

All patients transplanted for hepatitis C (HCV)-related cirrhosis will experience a recurrence of the viral disease on the liver graft with an accelerated course of the disease and a progression to advanced liver fibrosis in up to 50% of the patients at 5 years post-liver transplantation (1-3). HCV recurrence on liver grafts is a major concern as it is the first cause of liver graft loss and death in liver transplant recipient with HCV infection.

Several recent studies have shown efficacy and safety of direct acting antivirals (DAAs) in this special population in clinical studies as well as in real-life patients 'cohorts.

We report here three cases of patients who underwent a liver transplantation for hepatocellular carcinoma (HCC) on HCV-related cirrhosis who experienced an acute cellular rejection after the end of HCV therapy with DAAs.

1. Case reports (Table 1)

1.1. Case 1

Patient 1 was a 61-year-old lady with chronic HCV genotype 4 infection. She had previously been treated with pegylated interferon and ribavirin in 2009 without virological response and had developed an interferon-induced diabetes mellitus. She developed end-stage liver disease and hepatocellular carcinoma (HCC) in 2010 leading to a liver transplantation in Septembre 2011. Her immunosuppression was based on tacrolimus 1.5mg/day in monotherapy, stable since 2013. The trough levels were 5-7 ng/mL before the beginning of the antiviral treatment. A liver biopsy was performed in October 2015 showing features of chronic hepatitis compatible with HCV infection with fibrosis stage F2 (METAVIR.) The viral load at the beginning of the treatment was 5,654,133 IU/mL.

A treatment with ombitasvir/paritaprevir/ritonavir and Ribavirin (dose of 800 mg/day) was begun in May 2016 and ended 12 weeks later. The dose of tacrolimus was reduced during the treatment. At the end of the antiviral treatment the dose was reincreased at 1mg/day with trough levels of 8 ng/mL. Sustained virological response (SVR) was observed 12 weeks after completing the treatment.

A 5-year protocol liver biopsy was performed in October 2016 showing no rejection and a status compatible with post-HCV treatment. Fibrosis was graded METAVIR F1.

In February 2017 the patient experienced an elevation in ALT and AST levels at respectively 2 and 3-fold the upper limit of normal values (ULN). Tacrolimus trough levels were 3.5ng/mL with a good adherence. HCV-RNA PCR was again negative. Tacrolimus dose was increased at 1.5 mg/day with a control trough level at 7.2 ng/mL. However we observed a worsening of the liver tests. Mycophenolate Mofetil was added at 500 mg twice daily. A complete work-up was made including a new liver biopsy in March 2017 (Figure 1A) showing features of acute rejection (Banff P2D0V0.) The liver tests progressively improved and were normalized by the end of April 2017 and remained stable since now.

1.2. Case 2

Patient 2 was a 49 year-old man with a chronic HCV infection, genotype 3. He had experienced three lines of

Correspondence to : Geraldine Dahlqvist, Cliniques Universitaires Saint-Luc, 10 avenue Hippocrate 1200 Brussels Belgium.
E-mail : geraldine.dahlqvist@uclouvain.be.

Submission date : 24/01/2018
Acceptance date : 21/08/2018

Table 1. — **Patients' characteristics**

Patient	Age (Years)	Age at transplantation (Years)	Anti-HCV treatment	Tacrolimus trough levels before treatment (ng/mL)	Tacrolimus trough levels at the end of treatment (ng/mL)	Tacrolimus trough levels at SVR 3 (ng/mL)	Tacrolimus dosage before treatment (mg/day)	Tacrolimus dosage after treatment (mg/day)	ALT before treatment (UI/L)	ALT end of treatment (UI/L)	ALT at SVR 3 (UI/L)	Interval between end of treatment and rejection (months)
1	61	55	ombitasvir/paritaprevir/ritonavir and Ribavirin	6,6	12,1	8	1,75	1	32	10	57	6
2	49	46	sofosbuvir, daclatasvir and ribavirin	4,4	4,1	2	1,5	1,5	57	17	29	4
3	56	55	Ombitasvir/paritaprevir/ritonavir and dasabuvir	6	6	5,3	1,5	1	100	15	164	3

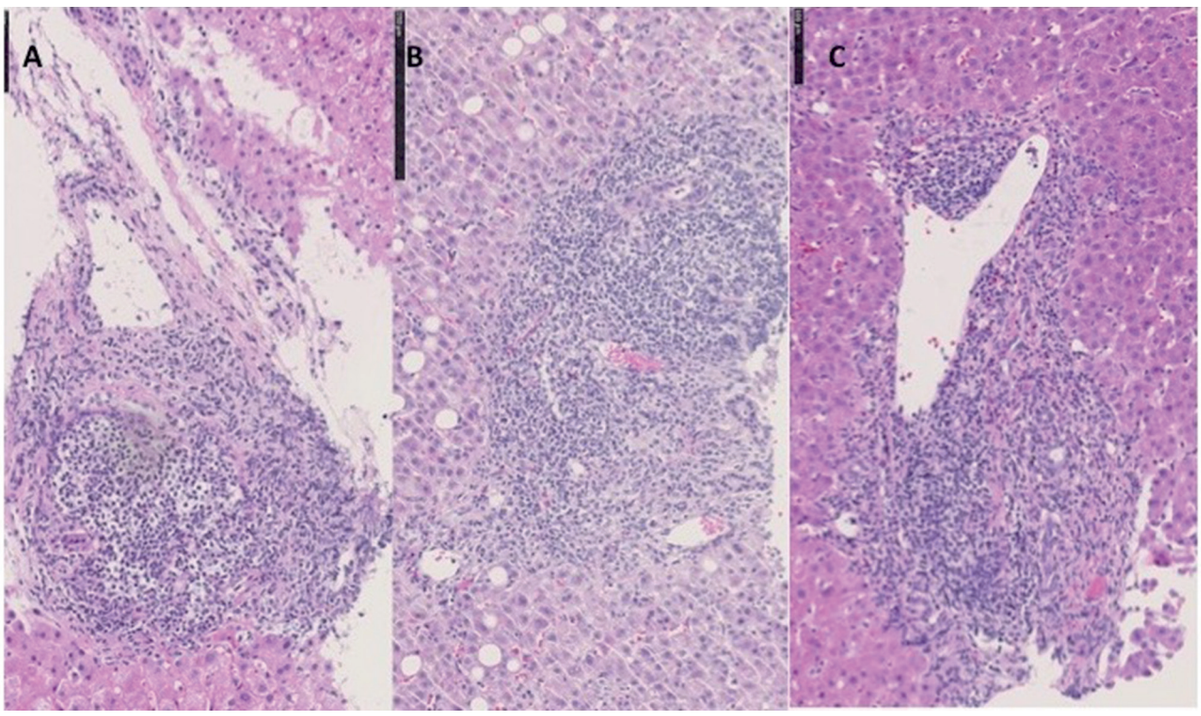

Figure 1 : A. Dense portal inflammation. Banff 2/7 (P2B0V0) ; B. Image of widespread lymphocytic cholangitis ; central perivenulitis and hepatocanalicular cholestasis ; C. Acute cellular rejection. Banff score 7/9 (P3B2V2).

treatment with pegylated interferon and ribavirin of 48 weeks with post-treatment recurrences. He developed an HCC on cirrhosis in 2007 and living donor liver transplantation was performed in 2013. He had a recurrence on the liver graft. The annual protocol biopsy performed in April 2015 showed a recurrence of hepatitis C, a METAVIR score F2 and no sign of rejection. The patient was treated from August 2015 to November 2015 with sofosbuvir, daclatasvir and ribavirin (1200 mg/day.) His immunosuppression was based on tacrolimus monotherapy with 1.5 mg/day and trough levels between 3.4 and 4.4ng/mL. His HCV viral load before treatment was 4,113,506 IU/mL.

He achieved SVR 12. The tacrolimus trough level at the end of the treatment was between 1.9 and 3.5 ng/mL. The liver tests were normal during and at the end of the treatment. They remained normal in monthly controls until March 2016 where an acute hepatitis occurred with cytolysis up to 9 ULN and elevated GGT and alkaline phosphatases up to 6 and 1.5 ULN, respectively. A complete work-up excluded HCV recurrence, HAV, HEV, CMV, EBV, HHV6, HBV infections. Antinuclear antibodies were positive at a titer of 1/640 with normal anti-actine values. There was no hypergammaglobulinemia. The patient did not take any new medication or herbal remedies.

A liver biopsy (Figure 1B) was performed in March 2016 showing feature of acute cellular rejection.

The dose of tacrolimus was increased up to 2 mg/day and mycophenolate mofetil at 1500mg/day was added after the biopsy with no improvement. The patient was then treated with boluses of methylprednisolone 1g during 3 days and then orally with 64 mg/day followed by lowering doses. The liver tests slowly improved. In August 2016, the methylprednisolone dose was tapered from 16 mg to 12 mg and the patient experienced a recurrence of high transaminases levels and very high GGT, up to 25 ULN. A new liver biopsy was performed in August confirming acute cellular rejection with no signs of chronic rejection. The dose of methylprednisolone was then re-increased to 16 mg/day until December 2016 followed by a slow response. It was then carefully reduced to 12 mg, then 8 mg with progressive normalization of the AST and ALT in May 2017. The tacrolimus trough levels were maintained between 5 and 8 ng/mL with tacrolimus daily dose increased at 3mg/day. The mycophenolate mofetil dose was diminished at 1000mg/day. The patient is now under triple immunosuppression with clinical and biochemical remission.

1.3. Case 3

Patient 3 was a 56 year-old man with a chronic HCV infection genotype 1b who developed cirrhosis diagnosed in 2011 complicated by a monofocal HCC of 3 cm in May 2013. He received a liver transplantation in January 2015 and HCV recurred on the graft with METAVIR F1 fibrosis on the 1-year protocol biopsy. The viral load was 2,344,393 IU/mL. Tacrolimus was given as monotherapy at a dose of 1.5 mg/day with a tacrolimus trough level of 6 ng/mL. Ombitasvir/paritaprevir/ritonavir and dasabuvir was started on July 2016 for 12 weeks. SVR was obtained in December 2016. During antiviral treatment tacrolimus was given at a dose of 0.5 mg/week. It was then increased at 1 mg/day, a lower dose than before treatment because of a kidney failure (creatine level 1.82 mg/dL), with trough levels of 3 ng/mL. The liver tests were completely normal at the end of the treatment and one month post-treatment but increased in December 2016 with AST et ALT at respectively 2.5 and 4 fold ULN. A liver biopsy was performed in December (Figure 1C) showing acute cellular rejection with a Banff score of 7/9 (P3B2V2.) Tacrolimus was increased up to 2 mg/day with trough levels 3.5 ng/mL and mycophenolate mofetil was added at 1000 mg/day the day after the liver biopsy. Following the modification in immunosuppressive treatment the liver tests normalized in a few weeks.

2. Discussion

We describe here the cases of three patients transplanted for HCC developed on HCV-related cirrhosis that were successfully treated with DAAs and who developed an acute cellular rejection more than three months after the end of a successful antiviral treatment.

HCV infection was until today the most common diagnosis in liver transplant recipients. As HCV recurrence is one of the leading causes for graft loss and death, HCV treatment after liver transplantation is a priority to avoid evolution to advanced fibrosis and to protect the patient against extra-hepatic complications such as diabetes mellitus, cardiovascular diseases or hepatitis C related renal diseases (1-3).

In the past, the standard of care for clearing HCV infection after liver transplantation was a 48 weeks peg-interferon and ribavirin regimen with poor tolerance and efficacy in only 30% of the patients. With the combination of peg-interferon-ribavirin and the first generation protease inhibitors, boceprevir or telaprevir, the SVR rate was achieved in 55-65% in genotype 1 infected patients (4-5). The treatment was feasible but difficult to manage due to of drug-to-drug interactions (DDI) and poor tolerance.

The new DAAs have opened the road to HCV eradication with good tolerance and less DDI in this difficult-to-treat population.

Experience of treating liver transplant recipients using an interferon-free antiviral regimen is emerging with more and more real-life experiences in mainly genotype 1, 3 and 4 patients (2,4,5,6).

The question of rejection occurring during or after HCV eradication in liver transplanted patients has already been discussed in the interferon-era. The immunostimulatory effect of interferon was then accused to potentially cause acute or chronic rejection but the results were contradictory (6,7). Kugelmas et al. suggested that improvement in a microsomal metabolic function after viral eradication with decreased in immunosuppressive drug trough levels predisposes patients to allograft rejection (8). An alternative theory for the relationship between HCV clearance and rejection is the increased immune response with higher levels of pro-inflammatory cytokines that characterizes responders to antiviral therapy (9). If the two last hypothesis are true, we should experience rejection episodes with DAAs as well.

The CUPILT study reported an SVR at week 12 in 96.4 % of the 137 studied patients treated with

sofosbuvir, daclatasvir +/- ribavirin. Only 2 patients (1.5 %) were reported with biopsy proven acute cellular rejection during the treatment but follow-up was limited to 12 weeks post-treatment (4).

The HCV-TARGET study reported 347 liver transplanted patients and 36 liver and kidney transplanted patients treated either with sofosbuvir/ledipasvir, sofosbuvir/daclatasvir or ombitasvir/paritaprevir/ritonavir + dasabuvir all +/- ribavirin. The SVR12 was achieved in 96.3 % of liver alone transplantation and 90.9 % in combined liver/kidney transplanted patients. There were 4 episodes of rejection reported in the liver transplant recipients, 2 during DAAs treatment and 2 after the treatment, one of the patient died, one patient kept graft dysfunction and the two others required steroid boluses and a combined immunosuppressive therapy (2).

Salcedo et al reported a cohort of 331 patients treated with daclatasvir and sofosbuvir or simeprevir +/- ribavirin. There was no episode of acute or chronic rejection but changes in immunosuppressive therapy were made in 32.5 % of the studied patients (6).

Although changes in immunosuppression are anticipated in ombitasvir/paritaprevir/ritonavir treatment due to the known interaction with CYP3A4, the other DAAs therapy should also be used with caution and immunosuppressors trough levels followed carefully to avoid rejection in patients with lower trough levels.

Smolders et al reported the cases of two patients in which tacrolimus dosage had to be increased during HCV treatment to maintain the target trough levels. The hypothesis is that CYP3A activity is reduced under the proinflammatory condition of HCV infection and when the virus is cleared, the activity of the enzyme is increased lowering the tacrolimus trough level (10).

Two of our patients were treated with the ombitasvir/paritaprevir/ritonavir combination and tacrolimus trough levels were followed cautiously during the treatment. In the third patient we have observed a decrease in tacrolimus trough levels at the end of the treatment. There were no signs of rejection during the first three months post-antiviral treatment, confirmed by normal liver tests in all patients and a protocol-liver biopsy in one of the patients. As our patients were transplanted for HCC the immunosuppressive regimen is based on minimal immunosuppression with tacrolimus monotherapy and trough levels between 3 and 5 ng/mL after 1 year post-transplantation to reduce the risk for HCC recurrence before as well as after the antiviral treatment.

In conclusion, HCV therapies have become easier to use, very efficacious and more available. With those three cases, including one with severe cortico-dependant rejection, we advocate for a close monitoring of tacrolimus and liver tests even a few months after the end of the treatment. Clinicians using DAAs after liver transplantation should be aware of the dynamics of tacrolimus levels during therapy and immunological changes that can occur even late after the end of treatment.

Conflict of interest statement

All authors confirm that there is no conflict of interest.

Financial support statement

All authors confirm that there is no financial support.

Authors' contributions

Geraldine Dahlqvist and Yves Horsmans contributed equally to the writing of this paper. All the authors contributed to the management and the diagnosis of the patients.

References

1. CRESPO G, MARINO Z, NACASA M, FORNS, X. Viral hepatitis in liver transplantation. *Gastroenterology*, 2012, **142** : 1373-1383.
2. SAXENA V, KHUNGA V, VERNA EC, LEVITSKY J, BROWN RS, HASSAN MA. *et al*. Safety and Efficacy of current DAA Regimens in Kidney and Liver Transplant Recipients with Hepatitic C : Results from the HCV-TARGET Study. *Hepatology*, 2017.
3. BERENGUER M, PRIETO M, RAYON JM, MORA J, PASTOR M, ORTIZ V. *et al*. Natural history of clinically compensated hepatitis C virus-related graft cirrhosis after liver transplantation. *Hepatology*, 2000, **32** : 852-858.
4. COILLY A, GOUGEROU-LEURENT C, DE LEDINGHEN V, HOUSSEL-DEBRY P, DUVOUX C, DI MARTINO V. *et al.* Multicentre experience using daclatasvir and sofosbuvir to treat hepatitis C recurrence – The ANRS CUPILT study. *J. Hepatol*., 2016, **65** : 711-718.
5. SALCEDO M, PRIETO M, CASTELLS L, PASCASIO JM, ALVAREZ JLM, FERNÁNDEZ I. *et al.* Efficacy and safety of daclatasvir-based antiviral therapy in HCV recurrence after liver transplantation. Role of cirrhosis and genotype 3. A multi-centre cohort study. *Transpl Int*., 2017 Jun 13. doi : 10.1111/tri.12999.
6. FÉRAY C, SAMUEL D, GIGOU M, PARADIS V, DAVID MF, LEMONNIER C, REYNÈS M, BISMUTH H. An Open Trial of Interferon Alfa Recombinant for Hepatitis C After Liver Transplantation : Antiviral Effects and Risk of Rejection. *Hepatology*, 1995 Oct, **22** : 1084-1089.
7. AHMAD J, DODSON SF , DEMETRIS AJ, FUNG JJ, SHAKIL AO. Recurrent Hepatitis C After Liver Transplantation: A Nonrandomized Trial of Interferon Alfa Alone Versus Interferon Alfa and Ribavirin. *Liver Transplantation*, 2001, **10** : 863-869
8. KUGELMAS M, OSGOOD MF, TROTTER JF, BAK T, WACHS M, FORMA L, KAM I, EVERSON GT. Hepatitis C Virus Therapy, Hepatocyte Drug Metabolism, and Risk for Acute Cellular Rejection. *Liver Transplantation*, 2003, **9**, 11 : 1159-1165.
9. SUGURU YONEDA,TAKEJI UMEMURA, YOSHIHIKO KATSUYAMA, ATSUSHI KAMIJO, SATORU JOSHITA, MICHIHARU KOMATSU *et al*. Association of Serum Cytokine Levels With Treatment Response to Pegylated Interferon and Ribavirin Therapy in Genotype 1 Chronic Hepatitis C Patients. *The Journal of Infectious Diseases*, 2011
10. SMOLDERS EJ, PAPE S, DE KANTER CTMM, VAN DEN BERG QP, DRENTH JPH, BURGER DM. Decreased tacrolimus plasma concentrations during HCV therapy : a drug-drug interaction or is there an alternative explanation ? *Int. J. of Antimicrpboam Agents*, 2017. **49** :379-382.