Caso clinico

Case reports

Epstein Barr virus infection reactivation as a possible trigger of primary biliary cirrhosis-like syndrome in a patient with multiple sclerosis in the course of fingolimod treatment

Riattivazione dell'infezione da Epstein Barr virus come possibile trigger di cirrosi biliare-like syndrome in un paziente con sclerosi multipla in trattamento con fingolimod

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INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized by the immune-mediated destruction of intrahepatic bile ducts, resulting in chronic liver injury and evolving to fibrosis, cirrhosis and liver failure. A distinguishing feature of PBC is the presence of antimitochondrial antibodies (AMA) in serum, found in 90%-95% of patients and less than 1% of normal controls [1, 2]. Multiple sclerosis (MS) is a complex immune-mediated inflammatory disease of the central nervous system affecting an estimated 2 million people worldwide [3]. Both MS and PBC are considered autoimmune diseases although the etiopathogenesis is still unclear. An association of both diseases has been previously described in sporadic case reports [4].

Fingolimod is the first orally administered disease-modifying drug and was approved in 2010 for the treatment of relapsing-remitting multiple sclerosis. Increased alanine aminotransferase (ALT) values >3 times the upper limit of normal (ULN) is reported to have occurred in 94 of 1,172 (8.0%) patients treated with fingolimod 0.5 mg. Furthermore, because fingolimod is a potent immunomodulator, an increased susceptibility to infections must be expected. To this regard one case of fatal disseminated primary varicella zoster (VZV) and one case of fatal herpes simplex (HSV) encephalitis have been described [5].

We describe the case of a 34-year-old man with relapsing-remitting MS since 2001 who present-

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ed an ALT and gamma-glutamyl transpeptidase (GGT) increase at the seventh month of fingolimod treatment and was diagnosed with PBC-like syndrome.

CASE REPORT

We describe the case of a 34-year-old man who had been diagnosed with relapsing-remitting multiple sclerosis in 2002 according to the Mc Donald criteria. He received therapy with interferon (IFN) beta 1a from 2002 to 2010, but the treatment was discontinued because ineffective according to the brain magnetic resonance imaging evidence. No alterations in liver chemistry were reported throughout this period. In March 2011 the patient started second-line treatment with fingolimod 0.5 mg once daily. The following clinical and laboratory evaluations were made before starting treatment: hematological evaluation with complete blood count, routine liver chemistry, IgM and IgG for varicella zoster virus (VZV), fundus examination and cardiology consultation with ECG. All results were normal and there was no evidence of impaired liver function. Serology results for VZV showed IgG positivity. Hematological and biochemistry parameters were evaluated monthly. After two weeks of therapy he presented a slight ALT elevation of 1.5 fold the ULN and GGT of 1.5 fold the ULN. Liver enzymes increased over the months up to the seventh month of treatment when values of 3-4 fold the ULN were reached (Figure 1). During this period, coincidentally with these findings, the patient felt mild pain in the right hypochondrium and abdominal discomfort after meals. Of note, about a month after treatment was started, an episode of fever with nausea, vomit-

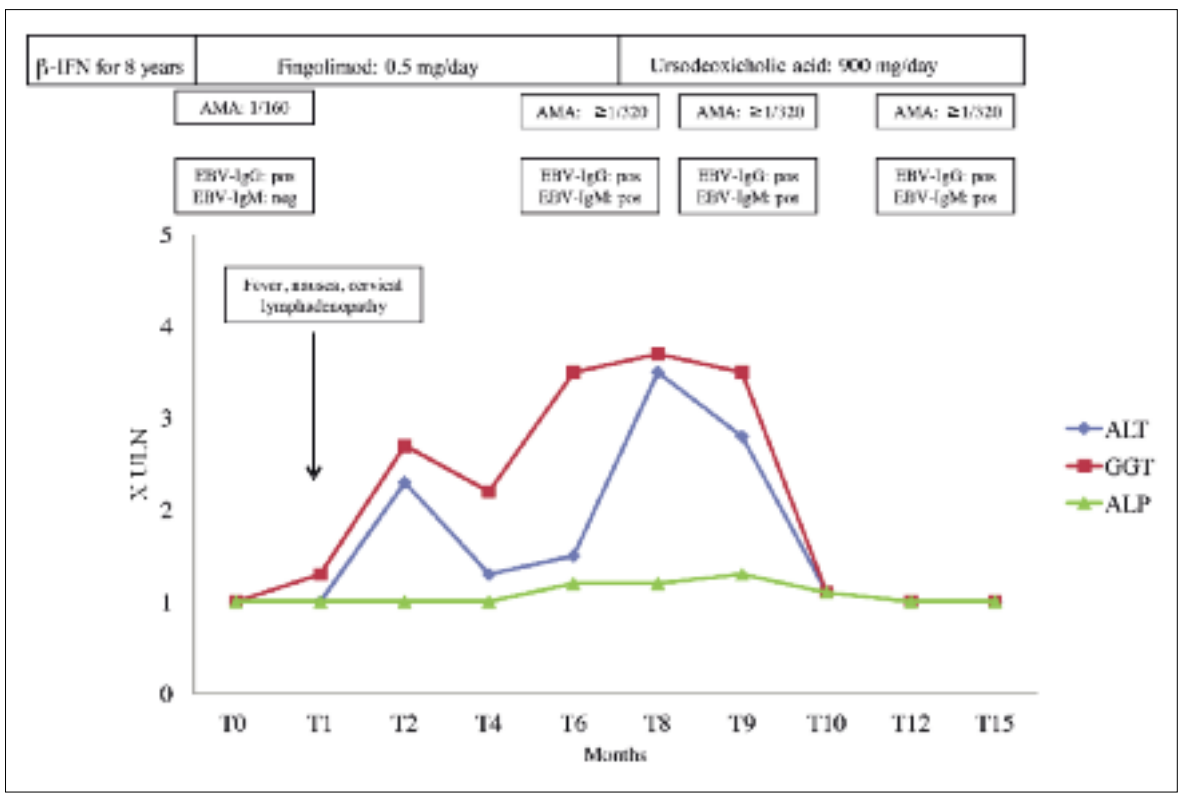

Figure 1 - Clinical course of a 34-year-old man with MS who received fingolimod treatment. The patient, with multiple sclerosis previously treated with beta-IFN for eight years, started fingolimod 0.5 mg/day and stopped at the 7th month of therapy due to ALT and GGT elevation. The liver chemistry test alterations were preceded by an episode of fever and lymphadenopathy associated with EBV-infection reactivation and positive IgM anti-EBV, followed by AMA titer increase. Fingolimod discontinuation and ursodeoxicholic administration led to liver enzyme normalization but with a persistence of IgM anti-EBV and AMA positivity. *ALT:* alanine aminotransferase; *GGT:* gamma-glutamyl transpeptidase; *ALP:* alkaline phosphatase; *EBV:* Epstein-Barr Virus serology; *AMA:* antimitochondrial autoantibodies; *ULN*: upper limit of normal.

Table 1 - Auto-antibody profile at the time of fingolimod discontinuation.

*AMA: ≥1/320	*ANA: neg	*ASMA: neg
^Anti-AMA-M2: neg	^Anti-M2-3E (BPO): pos	^Anti-Sp100: neg
^Anti-PML: neg	^Anti-gp210: neg	^Anti-LKM-1: neg
^Anti-LC-1: neg	^Anti-SLA/LP: neg	^Anti-Ro-52: neg

AMA: anti-mitochondrial antibodies; ANA: anti-nuclear antibodies ; ASMA: anti-smooth muscle autoantibodies were tested by indirect immunofluorescence (IIF)*. Nine different autoantibodies involved in autoimmune diseases were tested by a line immunoassay (LIA, Euroimmun)^: Anti-AMA-M2: anti-pyruvate dehydrogenase complex; Anti-M2-3E: anti-fusion protein of the E2 subunits of alpha-2-oxoacid dehydrogenases of the inner mitochondrial membrane; Anti-Sp100: anti-nuclear antigen; Anti-PML: anti-promyelocytic leukemia protein; Anti-gp210: anti-nuclear; Anti-LKM-1: anti-liver-kidney microsomes-1; Anti-LC-1: liver cytosolic antigen type 1; Anti-SLA/LP: anti-soluble liver antigen/liver-pancreas antigen; Anti-Ro-52.

ing, fatigue, upper abdominal pain and cervical lymphadenopathy occurred. Fingolimod therapy was discontinued at the seventh month and the patient was hospitalized for a complete liver function assessment. The physical examination revealed: pain on deep palpation of the right hypochondrium and increased liver size. Liver chemistry showed an ALT increase of 3.5 times the ULN, GGT 3.7 times the ULN, alkaline phosphatase (ALP) 1.3 times the ULN and normal bilirubin values. Total serum IgMs were slightly elevated (256 mg/dl). There was no evidence of hepatitis A, B or C virus infections. Serology for cytomegalovirus (CMV) and HSV was IgG-positive and IgM-negative. IgG and IgM anti-*Toxoplasma gondii* resulted negative. Interestingly, IgG and IgM were both positive for Epstein Barr virus (EBV). Serum liver-related autoantibodies revealed: anti-nuclear-antibody (ANA) negativity, anti-smooth muscle-antibody (ASMA) negativity, anti liver-kidney microsomal antibody (anti-LKM) negativity and, surprisingly, anti-mitochondrial-antibody (AMA) positivity ≥1:320. Liver US examination showed mild steatosis and slight hepatomegaly, no signs of bile duct obstruction and normal spleen size. A sample of the patient's serum taken prior to fingolimod administration was AMA-positive at a lower titer (1/160) and EBV-IgG-positive but EBV-IgM-negative. Treatment with ursodeoxycholic acid (15 mg/Kg/die) was started and after three months of therapy the liver function parameters normalized. After eight months of follow-up the patient presented normal liver enzymes, AMA positivity ≥1:320 and was still EBV-IgM-positive. The patient did not give consent for liver biopsy.

During fingolimod treatment the patient did not show any worsening in disability and no evidence of clinical or instrumental MS relapse. The liver enzymes, EBV serology and AMA titers over the course of fingolimod treatment and follow-up after fingolimod was discontinued are shown in Figure 1. Auto-antibodies were tested by indirect immunofluorescence and by a line immunoassay (Euroimmun Test Systems, Luebeck, Germany). The auto-antibody profile at the time fingolimod was discontinued is given in Table 1.

DISCUSSION

We describe a case of PBC-like syndrome that was unmasked, concomitantly or consequently to EBV reactivation, in a patient with relapsing multiple sclerosis who was receiving fingolimod treatment. The relationship between viral infections and the occurrence of autoimmunity and overt autoimmune disease is complex and intriguing. Pathogenetic mechanisms such as molecular mimicry, bystander activation, persistent virus infection and others have been investigated and proposed. However, accumulating evidence shows that infective agents can initiate, promote and even abrogate autoimmunity [6, 7-9]. There is strong epidemiological evidence that MS and PBC patients have a higher sero-prevalence of EBV antibodies, and increased levels of EBV DNA have been detected in the PBMC, liver tissue and saliva of PBC patients [6, 10, 11]. Although the epidemiological relationship is clear, the etiological role of EBV in both diseases is still uncertain. Recently an *in-vitro* study showed that EBV can exert its helper function for torque teno virus (TTV) replication, which is believed to play a role in the pathogenesis of MS [12].

In this clinical case, both the physicians and patient were unaware of AMA positivity before starting fingolimod, and at that time all liver

chemistry tests were normal. After about one month of fingolimod therapy, the patient presented an episode of fever, fatigue and cervical lymphadenopathy associated with an ALT and GGT increase that persisted until the seventh month when laboratory data showed anti-EBV-IgM positivity and higher AMA titers compared to the data before starting treatment. Fingolimod discontinuation and the administration of ursodeoxycholic acid led to liver chemistry normalization, but IgM anti-EBV positivity persisted for eight more months. The IgM anti-EBV seropositivity and possibly the fever, fatigue and cervical lymphadenopathy constitute the evidence for EBV reactivation. So we suppose that fingolimod immunosuppression was responsible for EBV-infection reactivation, which in turn triggered or unmasked latent PBC-like syndrome.

Chen recently reported the case of a 41-year-old man with relapsing-remitting multiple sclerosis who was receiving fingolimod and presented acute hepatitis with jaundice. The initial presumptive diagnosis was drug-induced liver injury (DILI) due to fingolimod, but subsequently, acute hepatitis E virus (HEV) was diagnosed [13]. HEV is usually responsible for acute hepatitis but it can evolve to a chronic disease in post-transplant immunosuppressed patients. Cases, even fatal, of herpes virus infection reactivation have been described in the course of fingolimod treatment, so it is conceivable that in our case, EBV reactivation was due to the immunomodulatory effect of fingolimod.

Recently, Pender proposed an interesting unifying multi-step hypothesis which correlates EBV infection to the development of autoimmune diseases. In this hypothesis, genetically determined CD8+ T-cell deficiency is a general feature of chronic autoimmune diseases and underlies their development by impairing CD8+ T-cell control of EBV infection, with the consequence that EBV-infected autoreactive B cells accumulate in the target organ, where they produce pathogenic autoantibodies and provide costimulatory survival signals to autoreactive T cells. The final evolution of these steps is the development of ectopic lymphoid follicles containing EBV-infected autoreactive B cells in the target organ [14].

Fingolimod is an antagonist of the sphingosine-1-phosphate receptor family (S1P1/3/4/5) that inhibits lymphocyte egress and their recirculation and presence in the central nervous system, trapping B and T lymphocytes in secondary lymphoid tissues. This reduces the lesion load and disability progression in multiple sclerosis. So fingolimod does not cause lymphopenia by depletion but by redistribution of lymphocytes. It has been demonstrated that in patients with PBC, there is a 100-fold to 150-fold increase in autoreactive CD4 PDC-E2–specific T cells and a 10-fold to15-fold increase in autoreactive CD8 PDCE2–specific T cell infiltrates in the liver and regional lymph nodes compared to the blood [1]. Although the mechanism of action is not completely known, it is reasonable to suppose, in our case, that by affecting T-cell recirculation, fingolimod determined, first, EBV-infection reactivation and, secondly, the clinical emergence of PBC-like syndrome, in accordance with the Pender hypothesis. It is difficult to establish in this case if the patient became symptomatic because of EBV reactivation or because of the immunomodulating action of fingolimod. However, after fingolimod was discontinued and ursodeoxycholic acid administered, the liver enzymes became normal but IgM-anti-EBV persisted for several months and the AMA titer increased.

It is not surprising that a clinical worsening of PBC occurs under immunosuppression. Recently a very rapid progression to cirrhosis was described in a patient with PBC and a gastric lymphoma who was receiving rituximab [15]. In our case, the sequence of EBV reactivation, liver enzyme alteration, AMA titer increase and the clinical improvement after fingolimod discontinuation and ursodeoxycholic acid administration makes the immunosuppression-viral reactivation-autoimmune disease sequence plausible.

Fingolimod is a promising and effective drug in the treatment of MS. Nevertheless, an increased susceptibility to infections, particularly related to the herpes viruses, and an elevation of liver enzymes in 8% of patients have been reported in the course of treatment. The causes of these hepatic effects are unknown and are generally attributed to drug-induced liver injury. Our case shows that some other underlying mechanism, such as immunosuppression, may have been responsible for a latent EBV-virus-infection reactivation and, consequently, the activation of PBC-like syndrome. Biological immunosuppressive drugs are changing the therapeutic armamentarium of onco-hematological, autoimmune, dermatological and neurological diseases but the risk of reactivation of occult in-

fections such as hepatitis B, herpes virus, JC virus and others must always be considered [16, 17].
In conclusion, this clinical case suggests to include a thorough liver assessment with complete virological and autoimmune screening in all patients with MS before starting fingolimod, and apply careful monitoring during therapy.
We would like to conclude by quoting the provocative statement by Zinkernagel: "If we know the infection, we call the disease immunopathologically mediated; if we do not recognize or know it, we call the disease autoimmune" [18]. However, if the infection directly infects autoreactive B cells, then the tissue damage may be mediated by autoimmune mechanisms rather than immunopathological mechanisms.

Disclosure of interest
The authors declare that they have no conflicts of interest concerning this article.

Keywords: Epstein Barr virus, primary biliary cirrhosis, multiple sclerosis, fingolimod, reactivation.

SUMMARY

Primary biliary cirrhosis (PBC) and multiple sclerosis (MS) are considered autoimmune diseases with a multifactorial aetiology which is thought to be due to a combination of genetic predisposition and environmental triggers.
An association of both diseases has been previously described in sporadic case reports. Fingolimod, an antagonist of the sphingosine-1-phosphate receptor family (S1P1/3/4/5), is a promising and effective drug in the treatment of MS. Here we describe a case of PBC-like syndrome that was unmasked, concomitantly or consequently to Epstein Barr virus (EBV)-infection reactivation, in a 34-year-old male patient with relapsing-remitting multiple sclerosis who was receiving fingolimod treatment.

RIASSUNTO

*La cirrosi biliare primitiva (PBC) e la sclerosi multipla (MS) sono entrambe considerate malattie autoimmuni ad eziologia multifattoriale cui concorrono condizioni genetiche predisponenti e fattori scatenanti ambientali. Un'associazione di entrambe le malattie è stata precedentemente descritta in sporadici casi clinici. Fingolimod, antagonista recettori della sfingosina 1-fosfato (S1P1/3/4/5), è un promettente ed efficace farmaco per il trattamento della sclerosi multipla. Descriviamo un caso di cirrosi biliare-*like syndrome *insorto in concomitanza o conseguentemente alla riattivazione dell'infezione da Epstein Barr (EBV) in un paziente con sclerosi multipla recidivante-remittente in trattamento con fingolimod.*

REFERENCES

[1] Lindor K.D., Gershwin M.E., Poupon R., Kaplan M., Bergasa N.V., Heathcote E.J. Primary biliary cirrhosis. *Hepatology* 50, 291-308, 2009.
[2] Poupon R. Primary biliary cirrhosis: a 2010 update. *J. Hepatol.* 52, 745-758, 2010.
[3] Compston A., Coles A. Multiple sclerosis. *Lancet* 359, 1221-1231, 2002.
[4] Pontecorvo M.J., Levinson J.D., Roth J.A. A patient with primary biliary cirrhosis and multiple sclerosis. *Am. J. Med.* 92, 433-436, 1992.
[5] Cohen J.A., Chun J. Mechanisms of fingolimod's efficacy and adverse effects in multiple sclerosis. *Ann. Neurol.* 69, 759-777, 2011.
[6] Selmi C., Papini A.M., Pugliese P., Alcaro M.C., Gershwin M.E. Environmental pathways to autoimmune diseases: the cases of primary biliary cirrhosis and multiple sclerosis. *Arch. Med. Sci.* 7, 368-380, 2011.
[7] Fujinami R.S., von Herrath M.G., Christen U., Whitton J.L. Molecular mimicry, bystander activation or viral persistence: infections and autoimmune disease. *Clin. Microbiol. Rev.* 19, 80-94, 2006.
[8] Christen U., Hintermann E., Holdener M., von Herrath M.G. Viral triggers for autoimmunity: is the 'glass of molecular mimicry' half full or half empty? *J. Autoimmun.* 34, 38-44, 2010.
[9] Sallie R. Replicative homeostasis II: influence of polymerase fidelity on RNA virus quasispecies biology: implications for immune recognition, viral autoimmunity and other "virus receptor" diseases. *Virol. J.* 2, 70, 2005.
[10] Almohmeed Y.H., Avenell A., Aucott L., Vickers

M.A. Systematic review and meta-analysis of the sero-epidemiological association between Epstein Barr virus and multiple sclerosis. *PLoS One* 8, 4, 2013.
[11] Rigopoulou E.I., Smyk D.S., Matthews C.E., et al. Epstein-Barr virus as a trigger of autoimmune liver diseases. *Adv. Virol.* 2012, 98747, 2012.
[12] Borkosky S.S., Whitley C., Kopp-Schneider A., zur Hausen H., de Villiers E.M. Epstein-Barr virus stimulates torque teno virus replication: a possible relationship to multiple sclerosis. *PLoS One* 7, 2, 2012.
[13] Chen E.Y., Baum K., Collins W., et al. Hepatitis E masquerading as drug-induced liver injury. *Hepatology* 56, 2420-2423, 2012.
[14] Pender M.P. CD8+ T-Cell deficiency, Epstein-Barr virus infection, vitamin D deficiency, and steps to autoimmunity: a unifying hypothesis. *Autoimmune Dis.* 2012, 189096, 2012.
[15] Tajiri K., Tsuneyama K., Miyazono T., Kawai K., Minemura M., Sugiyama T. A case of primary biliary cirrhosis that progressed rapidly after treatment involving rituximab. *Case Rep. Gastroenterol.* 7, 195-201, 2013.
[16] Yeo W., Chan T.C., Leung N.W. et al. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J. Clin. Oncol.* 27, 4, 605-611, 2009.
[17] Riad H., Shaikha S.A., Ansari H.A., Naama K.A., Sada H.A., Mansoori M.A. Hepatitis and lupus-like syndrome during Infliximab therapy for psoriasis. *Case Rep. Dermatol.* 5, 2, 219-224, 2013.
[18] Zinkernagel R.M. Antiinfection immunity and autoimmunity. *Ann. N.Y. Acad. Sci.* 958, 3-6, 2002.