Post Transplantation Lymphoproliferative Disorder (PTLD) Presenting as Biliary Duct Obstruction

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Abstract: After adult liver transplantation (LT), post-transplant lymphoproliferative disorder (PTLD) is an uncommon but serious complication of immunosuppression (IMS) in presence of an acute or latent EBV infection. The clinical presentation of this disease is aspecific, and, after LT, it may mimic anastomotic bile duct stricture.

We report the cases of 2 adult patients who developed, 3 months and 8 years after OLT, an EBV-associated PTLD with diffuse intrinsic infiltration of bile duct mimicking anastomotic biliary stricture. In the absence of liver or hilar nodes involvement, percutaneous biopsies were non contributive and the diagnosis were made by surgical biopsy. After reduction of IMS and Rituximab treatment, both patients are alive without recurrence 5 and 6 years after diagnosis.

In conclusion, PTLD is one of the differential diagnosis of biliary tree obstruction after OLT. Diagnosis requires surgical biopsies and treatment consists in IMS reduction and Rituximab.

INTRODUCTION

Post-transplant lymphoproliferative disorder (PTLD) which occurs in about 10% of pediatric liver allograft recipients, is an uncommon complication after adult liver transplantation (LT) (0.5-4.3%) [1-4]. The majority of PTLD are linked to acute or latent Epstein-Barr virus (EBV) infection and the main risk factors are both the type and the intensity of immunosuppression (IMS). The rare cases of EBV-negative PTLD tend to occur later after the transplant, and behave more aggressively [2].

The spectrum of presentation of PTLD varies from localized to disseminated involvement and may concern lymph nodes, allograft organ or sites of surgical intervention [1-5].

We report the cases of 2 adult patients who developed, 3 months and 8 years after OLT, an EBV-associated PTLD mimicking anastomotic biliary stricture.

CASE REPORTS

Patient 1

In April 2002, a 52-year-old man underwent LT for alcoholic cirrhosis and hepatocarcinoma (HCC). He was CMV and EBV IgG positive, and IgM negative. The initial IMS regimen consisted in 20 mg of Basiliximab on day 1 and 4, mycophenolate mofetil (MMF) 250 mg bid and Cyclosporin (CyA) with target through levels of 200 µg/l.

Two weeks after LT, the patient developed an asymptomatic CMV infection treated with intravenous ganciclovir (5 mg/kg bid).

Three months after LT, the patient presented with a cholestasis (GGT 5N, alkaline phosphatase 1.5N) and bilirubin 61µmol/l. Abdominal ultrasound, cholangio-RMI, CT-scan and percutaneous cholangiogram showed a long stenosis of the bilio-biliary anastomosis and an enlargement of the intrahepatic biliary ducts (**image 1**). The patient underwent surgical resection of the stenosis with confection of a jejuno-biliary anastomosis. Histological analysis showed a peribiliary and biliary infiltration by numerous B lymphoblasts and some lymphocytes (**image 2**). Immunohistochemical

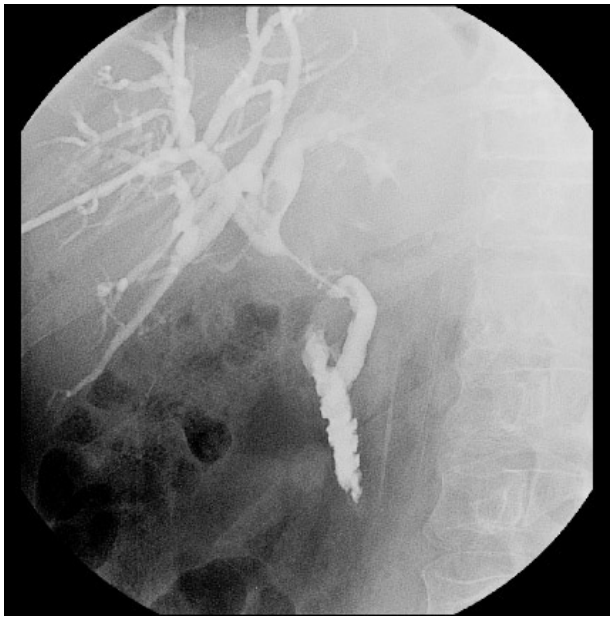

Image 1: **Patient 1**: percutaneous cholangiogram showing an enlargement of the intrahepatic biliary ducts

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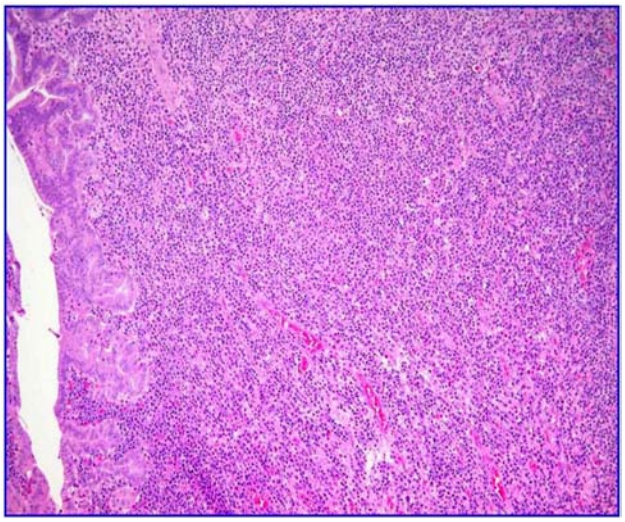

Image 2: Patient 1: Histological analysis showing a peri-biliary and biliary infiltration by a PTLD of polymorphic B lymphoblasts.

stains were positive for CD-20 (B-cell marker) and negative for CD-3 (T-cell marker). The ratio of kappa/lambda immunoglobulines was 10/1. Research of immunoglobulines genes rearrangement by PCR identified one clonal population of B cells. EBV was positive by EBER, EBNA2, EBNA3 and viral DNA by real time PCR was strongly positive in the biopsy (1,2 mio EBV copies per microgram of DNA) but undetectable in the serum. The diagnosis of an EBV-associated B-cell polymorphic PTLD was made [6,7]. A thoraco-abdominal CT-scan excluded other tumoral localizations. MMF was discontinued and CyA decreased (through level of 80 µmol/l) and rituximab was administrated weekly for 1 month followed by 4 monthly injections of 375 mg/m^2.

Six years after the diagnosis, the patient was treated with CyA (through level 50 µg/l) and MMF (250 mg bid). Physical examination, total body CT-scan, liver biopsy and liver function have been normal for 6 years without evidence of rejection or PTLD recurrence.

Patient 2

In February 1995, a 24 year-old man underwent LT for primary sclerosis cholangitis. He was CMV negative and EBV IgG positive. Initial IMS consisted of CyA (through level 200-336 µg/l during the first year, below 100 µg/l during the second year) methylprednisolone (20 mg/d initially, stopped 17 months after LT), and azathioprine (200 mg/d initially, 75 mg/d at the end of the first year, 50 mg/d from the second to the fourth year).

In January1998, with low levels of IMS (CyA through level below 100 µg/l, Azathioprine 50 mg/d), the patient presented a moderate acute rejection successfully treated by switching from CyA to Tacrolimus (through levels 10-15 µg/l). Since July 1998 he had fluctuating levels of ALT and AST (2-3 N) with a constant increased GGT (7N) and alkaline phosphatase (2N). Liver biopsy performed in 1998-99 showed portal focal peri-biliary fibrosis and bile ducts proliferation, suggesting either a biliary obstruction or a recurrence of primary cholangitis. Liver tests normalized after switching from Azathioprine 50 mg/d to MMF 500 mg bid. In February 2002, 7 years after LT, GGT and alkaline phosphatase increased again (7 and 2N, respectively) with a left intra-hepatic bile duct enlargement. Biopsy showed dystrophic biliary ducts, minimal neoductular proliferation with concentric peri-biliary fibrosis and focally extensive portal fibrosis and cholangiography showed a severe stenosis of the bilio-digestive anastomosis, which was treated by antibiotics, dilatation and drainage. The liver tests improved and biliary obstruction resolved after two months of drainage. Eight months after the drainage-tube removal, the patient presented again with elevated liver enzymes. Biopsy showed a portal, peri portal and lobular non blastic lymphocyte aggregates with portal fibrosis. A new cholangio-RMI disclosed a new, important, stenosis located on the bile ducts convergence. Laparotomy performed in November 2003, revealed an inflammatory thickening of the common, right and left bile ducts walls with obstruction of the common bile duct, and new anastomosis was performed at the roof of the biliary convergence. Histological analysis showed a peri-biliary and biliary infiltration by polymorphic B lymphocytes, lymphoblasts and plasmocytes (**image 3**), CD 79a and CD20 positive. Repartition of lambda and kappa immunoglobulines was homogenous by immunomarkers, and research of immunoglobulines genes rearrangement by PCR and Southern Blot did not identify clonal population of B cells. Some cells were EBV positive by EBER, EBNA2, EBNA3. EBV DNA by real time PCR was positive in the bile duct but negative in the serum. The diagnosis of polymorphic EBV-associated B-cell PTLD was made [6]. Neck, thoracic and abdominal CT-scan as well as bone marrow biopsy excluded extension of the disease. Quantification of EBV DNA by real time PCR in the serum was negative. MMF was discontinued and Tacrolimus was decreased but this was complicated by a rejection (grade 6 according to Banff classification) [8]. Tacrolimus was switched to rapamycine (target levels of 8 mg/l) and 20 mg of methylprednisolone were added. Liver tests were progressively improved. One month after surgery rituximab was administrated as 4 weekly injections of 375 mg/m^2 without complication. CT-scan which were, cholangio-MRI and PET-CT scan performed every year during five years did not show tumoral recurrence.

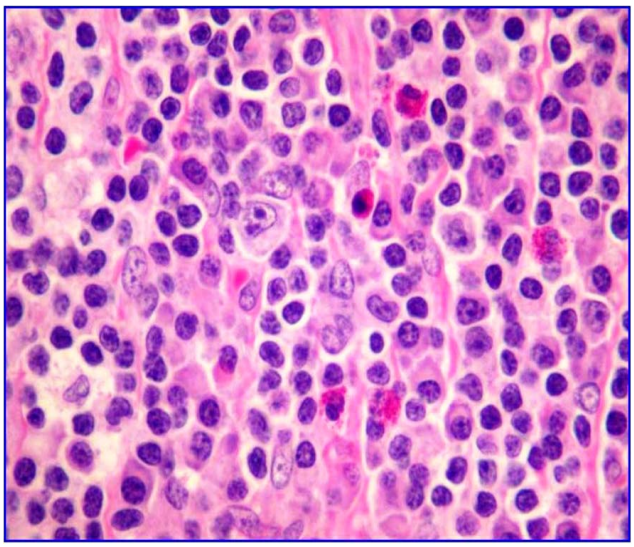

Image 3: Patient 2: Histological analysis showing a peri-biliary infiltration by a PTLD of polymorphic B lymphoblasts, CD20 positive in the resected bile duct.

DISCUSSION

Biliary complications occur in about 15% of patients after LT [9]. Strictures that develop during the first three post operative months are mainly anastomotic, due to local ischemia at the anastomosis or suturing technique. The late biliary strictures and obstruction may be anastomotic, often isolated and short. They may be hilar or intrahepatic, usually more diffuse and severe, evoking the differential diagnosis of arterial occlusion, prolonged cold ischemic time, CMV infection, ABO-incompatible transplantation or recurrence of primary sclerosing cholangitis [9].

We report here the cases of 2 adult patients who developed, 3 months and 8 years after LT, an EBV-associated PTLD mimicking anastomotic biliary stricture. Compression of the bile duct by hilar nodes has been reported in primary lymphoma occurring in non transplant population [10-12]. After liver transplantation, PTLD presenting as a localized single hilar tumor with obstructive jaundice has been described [1, 4, 13-19] in 20 patients. However, diffuse intrinsic infiltration of bile duct by a PTLD is extremely rare: in the series of 16 post liver transplantation PTLD described by Duvoux *et al.* only one concerned the biliary tract [1].

As PTLD lesions of our patients did involve neither the hepatic parenchyma nor hilar nodes, percutaneous liver biopsies were not contributive and diagnosis was unsuspected until surgery. Endoscopic biopsies performed before dilatation and stenting of the biliary tract could probably have been more informative.

The majority of PTLD are secondary to EBV which may cause an uncontrolled proliferation of B-lymphocytes in the absence of an effective cytotoxic T cells response. EBV genome is present in 68 to 100% [1,3,5] of adult PTLD, but EBV DNA research by PCR in the serum is often negative.

Despite the fact that surgical biopsy is often the only way to make the diagnosis, the effective treatment of biliary PTLD does not rely on surgery but on the reduction or the complete discontinuation of IMS [3]. To decrease the risk of rejection, the switch from Calcineurine inhibitors (CNI) to mTOR inhibitors should be preferred to complete IMS withdrawal. Immunosuppression by Sirolimus or everolimus, which are active through inhibition of the mTOR pathway, seems to inhibit the proliferation and survival of the PTLD cell-line derived from recipients PTLD [20, 21]. This antiproliferative action could be effective not only in preventing graft rejection but also in controlling lymphocytes proliferation.

In addition to IMS modification, management of PTLD includes Rituximab, a chimeric mouse-human anti CD20 antibody, directed against the surface antigens of both the normal and malignant B-lymphocytes, mediating a complement-dependant cellular lysis with antibody-dependant cellular cytotoxicity. The use of Rituximab was reported in the treatment of PTLD after solid organ transplantation [22, 23] with complete remission up to 87.5% [23]. Since 2000, five articles reported the use of rituximab in 5 adult and 7 pediatric patients who developed PTLD after LT [24-28]. Treatment was well tolerated and led to complete remission in 4 adult patients [27] and 6 pediatric patients with a follow-up going from 10 weeks to 3 years.

CONCLUSION

PTLD is a rare but serious complications of immunosuppression in presence of an acute or latent EBV infection. The clinical presentation of this disease is aspecific, and, after OLT, it may mimic anastomotic or nonanastomotic bile duct stricture. Therefore, PTLD is one of the differential diagnoses of biliary tree obstruction after OLT. In this case, laparotomy and surgical biopsies are the only methods to confirm the diagnosis.

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