 Prague Medical Report / Vol. 110 (2009) No. 3, p. 214–221

Allograft Hepatitis after Liver Transplantation for Epithelioid Haemangioendothelioma

Honsová E.[1], Gottfriedová H.[2], Oliverius M.[3], Trunečka P.[2]
[1]Department of Clinical and Transplant Pathology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic;
[2]Department of Hepatology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic;
[3]Department of Transplant Surgery, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Received January 23, 2009; Accepted April 30, 2009.

Key words: Epithelioid haemangioendothelioma – Liver – Transplantation – Allograft hepatitis

Abstract: Primary hepatic epitheloid heamangioendothelioma (EHE) is a rare neoplasm of vascular origin. We present a retrospective study of 6 patients with EHE treated by liver transplantation that were monitored clinically for more than 3 years and had protocol biopsy samples taken at 1, 3, 5, 7, and 10 years post-transplant. None of the patients suffered from any form of viral or autoimmune hepatitis before or after the transplantation. Two patients had lung metastases detected by preoperative imaging. All tumours showed factor VIII, CD31, and CD34 strong positive staining. In 5 of the 6 transplant recipients the protocol graft biopsies showed chronic non-specific hepatitis with slowly progressive periportal fibrosis that appeared during the 3rd post-transplant year. The septal fibrosis was diagnosed in the 6^{th} and 10^{th} year after transplantation. Liver tests did not reflect either the presence or the degree of inflammation or fibrosis and have remained normal. In retrospect, we consider that our recipients most probably developed alloantigen dependent inflammatory and fibrotic damage to their liver grafts. All six recipients are still alive for a median survival time of 95.1 month (range 44 months to 132 months), with good graft function, and without recurrence of the tumor. The lung metastases in 2 of the 6 patients have remained unchanged for 10 and 12 years retrospectively.

This study was supported by the Institute for Clinical and Experimental Medicine institutional grant MZO 00023001.

Mailing Address: Eva Honsová, MD., PhD., Department of Pathology, Institute for Clinical and Experimental Medicine (IKEM), Vídeňská 1958/9, 140 21 Prague 4, Czech Republic; Phone: +420 236 055 231; Fax: +420 236 053 076; e-mail: eva.honsova@ikem.cz

Introduction

Epithelioid haemangioendothelioma (EHE) is a rare neoplasm of vascular origin, often slowly progressive but with unpredictable malignant potential. In the liver it is usually found in the young and middle-aged adults, with a higher prevalence of women [1]. The clinical manifestation is usually non-specific, and includes right upper quadrant abdominal pain, weight loss, Budd-Chiari syndrome, or occasionally, the patients are entirely asymptomatic [2]. Although this neoplasm is frequently slowly progressive its clinical course can be highly unpredictable. It does not respond to chemotherapy or radiotherapy and for that reason the surgical resection has been the recommended method of treatment. Unfortunately in the majority of cases the primary liver EHE tends to be multifocal, which precludes complete resection, and thus orthoptic liver transplantation (OLT) is the only worthwhile treatment for these patients and for those with extensive liver involvement offering them a chance of long survival [3]. Liver histology in the protocol biopsy samples from the allografts has rarely been normal even if the liver function tests were normal. Chronic hepatitis (CH) is the most common histological finding, particularly in the late posttransplantation biopsy samples and occurs in up to 40% of the liver samples taken more than one year after the transplantation [4, 5].

Material and Methods

Patients

Between 1996 and 2006 there were 514 liver transplantations performed at our centre. Six of the recipients were adults with multifocal EHE (4 women, 2 men, age 35–51 years). Two women (37- and 51-year-old) had lung metastases detected by preoperative imaging. None of the patients suffered from viral hepatitis or autoimmune disease. Their postoperative course was unremarkable.

Liver histology

Native liver specimens were evaluated according to the routine protocol of our laboratory and the investigation included immunohistochemical staining with CD31, CD34, factor VIII, and CK19. The posttransplant liver biopsy samples were obtained according to the protocol 1, 2, 3, 5, 7 and 10 years post-OLT and routinely stained with H&E, van Gieson, orcein, PAS with diastase pre-treatment and with Perl's method. In retrospective assessment presence of B and T lymphocytes was evaluated using immunohistochemical staining with anti-CD20 and anti-CD3 antibody. Chronic hepatitis was characterized by a predominantly portal-based mononuclear inflammatory infiltrate with interface hepatitis in the absence of the biliary or vascular abnormalities that would have been typical of the rejection changes or the vascular or biliary complications. The inflammatory changes and the liver fibrosis were graded as mild, moderate or severe using modified hepatitic activity index [6].

Immunosuppression

After OLT all patients were receiving triple immunosuppressive therapy including corticosteroids, azathioprine (AZA) or mycofenolate mofetil (MMF) with either cyclosporine or tacrolimus as the primary immunosuppressive drug. Cyclosporine A was used as the primary calcineurin inhibitor in 4 patients and tacrolimus in 2 patients. Cyclosporine A was administered as the initial treatment orally, 3–6 mg/kg, having been given in two daily doses. Later doses were adjusted according to the blood levels of the drug. Tacrolimus was administered orally 12 h after the OLT as a dose of 0.05–0.1 mg/kg and then every 12 hours, the later doses were adjusted according to the blood levels. AZA was given at a dose of 1 to 2 mg/kg/day. In 3 patients MMF was used instead of AZA in a dose of 1–2 g/day. Due to leucopenia AZA had to be discontinued in one patient and MMF in another. Prednisone was started at a dose of 0.5 mg/kg/day and continued as a maintenance dose 5–10 mg daily. It was discontinued in 3 patients after 6, 33 and 37 months respectively.

Results

Native liver specimen histology

Five patients had multiple white or grey-white hard tumour nodules varying in size from 5 mm to 150 mm in diameter throughout the entire liver, the 6th patient had an extensive single lesion involving both liver lobes. Microscopically, the majority of tumor nodules displayed an ill-defined growth pattern with infiltrative margins, the tumor cells extending into the liver sinusoids, the hepatic venules and the radices of the portal vein (Figure 1). The hepatic arteries have not been invaded. All tumours showed strongly positive staining for factor VIII, CD31, and CD34; all were CK19 negative (Figure 2). Presence of necrotic and fibrotic areas and the cellular pleiomorphism are summarized in Table 1. Liver parenchyma outside the tumor nodules did not show any significant changes. None of the patients suffered from infectious hepatitis or autoimmune disease.

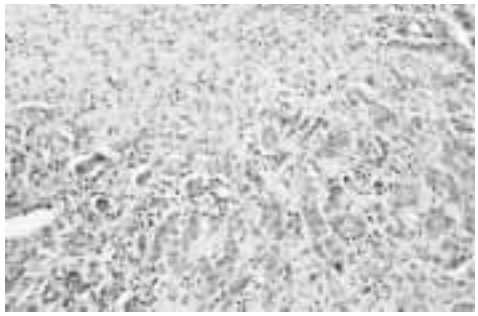

Figure 1 – Epithelioid hemangioendothelioma in native hepatectomy specimen. Sclerosed part of the tumour (top) and tumor cells infiltrating the sinusoids (original magnification ×100, H&E).

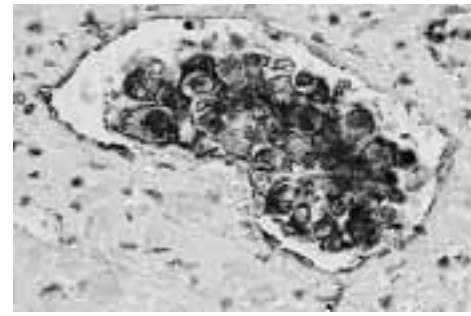

Figure 2 – Detail of intraluminal cluster of tumor cells strongly positive with anti-CD31 (original magnification ×400).

Clinical presentation prior to transplantation
Four out of 6 patients presented with abdominal pain and one of them also suffered from weakness and weight loss. The patient with single extensive liver mass only complained of pruritus and one male patient was entirely without any symptoms. His tumour was found on examination prior to his serious involvement in a sport activity.

Posttransplant liver biopsy samples
Early after OLT, one recipient had an episode of mild acute cellular rejection, 3 others suffered from repeated episodes of mild acute rejection. One male patient had an episode of severe cellular rejection due to noncompliance and withdrawal of immunosuppressive therapy in the 4th year after transplant. The remaining male recipient did not undergo a protocol biopsy at our centre, and has been treated in Slovakia.

A total of 23 protocol biopsy samples from 5 recipients were obtained since their transplantation and 22 were retrospectively examined by the same consultant pathologist (EH); the 23rd sample was not examined because of its small size. 20 biopsy samples were obtained according to protocol at 1, 3, 5, 7 and 10 years post transplantation and 3 others were evaluated in the 2nd or 4th year post OLT. The graft biopsy samples showed mild lobular and portal tract inflammation with focal interface activity and slowly progressive periportal and/or septal fibrosis. In 4 recipients the histological features of hepatitis were noted during the first year and subsequently there were found in the majority of the following protocol biopsy samples (19/22). The inflammatory activity was classified as mild or moderate in all cases (Figure 3). In all 19 biopsy samples the inflammatory changes were focal and were found in the portal tracts as well as in the lobules, mainly in the centrilobular areas (Figure 4). The so-called centrilobular drop-out of hepatocytes displayed focal pattern. There were no centrilobular circular lesions, and plasma cells were

Table 1 – Clinical and histological data of six patients who underwent OLT for EHE

Gender	Age	Tumor	Tumor necrosis	Tumor fibrosis	Nuclear atypia	Metastases	Survival time
f	51	Large single mass	extensive	marked, extensive	marked	lung, lymph node	12
f	42	Multifocal	no	moderate	mild	no	11
f	37	Multifocal	no	marked	marked	lung	10
m	35	Multifocal	focal	moderate	moderate	no	7
f	35	Multifocal	focal	marked	moderate	no	7
m	39	Multifocal	focal	marked	marked	no	4

Age – at time of transplantation; Survival time – years since transplantation

not a prominent component of the inflammatory infiltrate. All 5 recipients had aggregates of lymphocytes in the portal tracts the majority of which were CD3 positive, the same as the cells in the inflammatory infiltrate of the interface zones. CD20 positive B cells were also present but in small numbers. Scattered isolated eosinophils were identified in some portal tracts and in the areas of centrilobular inflammation.

Periportal fibrosis appeared within the first post-OLT year in 1 recipient; in the remaining 4 this occurred in the 3rd year or later. The septal fibrosis was diagnosed in the 6th and 10th year post OLT year in 2 patients. The morphological changes in the post transplant protocol biopsy samples are summarized in Table 2.

Post transplant clinical data

All recipients are still alive with good graft function, without recurrence of the disease, (median survival time 95.1 month, range 44–132 months). The lung metastases have remained stable, without any signs of progression. Four out of 6 recipients, all women, have now been monitored for 7, 10, 11 and 12 years.

Discussion

Primary hepatic EHE is a rare tumor of vascular origin. The first series of 32 cases of EHE in the liver was described by Ishak et al. in 1984 [2]. There was a predominance of women (62%) among the patients in this series. In histology, the tumor had often been misdiagnosed as a sclerosing carcinoma of bile duct origin. Clinically, the differential diagnosis was usually difficult due to the absence of specific symptoms and the lack of abnormality of the liver function.

The clinical manifestations of EHE are usually right upper abdominal quadrant pain, weight loss, weakness, and the symptoms of Budd-Chiari syndrome but

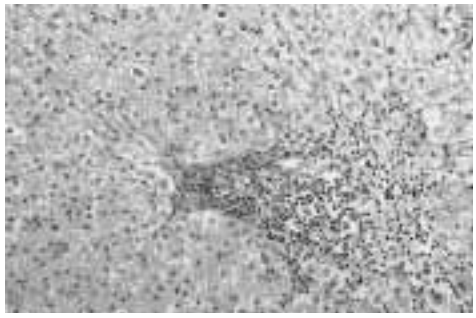

Figure 3 – Protocol biopsy sample 6 years after OLT, (liver function tests normal). Portal tract contains infiltrate of lymphoid cells associated with interface hepatitis (original magnification ×200, H&E).

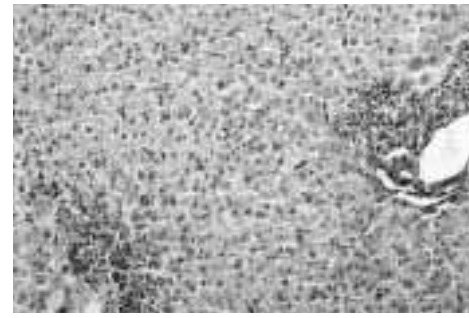

Figure 4 – Protocol biopsy (7 years post-transplant) portal tract shows mild inflammation and focal interface activity, an area of perivenular inflammation is associated with focal centrilobular necrosis (original magnification ×200, PAS with diastase pre-treatment).

approximately a quarter of the patients in the published series were asymptomatic at the time of diagnosis [7]. The results of the liver imaging are usually not suggestive of a vascular tumor and are often regarded as being more in favour of metastatic carcinoma [8] and the definitive diagnosis of EHE can only be made histologically. It is assumed that the true incidence of hepatic EHE is probably underestimated because some of these tumours could have been misdiagnosed as a metastatic carcinoma or cholangiocarcinoma [1]. In the Ishak's series the reporting pathologists made correct diagnosis only in 25% of the cases and in the review by Mehrabi 60% to 80% of patients with EHE had been initially misdiagnosed [6]. It is not clear whether the diagnostic conclusions have always been made on the basis of routine H&E staining alone. If it were a case, the solid clusters of tumor cells with the cytoplasmatic vacuoles could have been regarded as very suggestive of metastatic carcinoma or cholangiocarcinoma. Immunohistochemical staining could be very helpful in arriving at a correct diagnosis. Neoplastic cells of EHE are positive for the markers of endothelial differentiation (CD31, CD34, factor VIII). Weibel-Palade bodies could be identified ultrastructurally, but such an examination is expensive and time consuming. Even when the correct histological diagnosis has been made there are no reliable clinical or histological criteria by which the progression of the disease could be predicted [9] and thus it is difficult to design the best therapeutic algorithm for this neoplasm. Since it does not respond to chemotherapy or radiotherapy and complete surgical resection may not be achievable, as the majority of the hepatic EHEs are multifocal, liver transplantation is the only remaining method of treatment that can offer a possibility of a long-term cure. Involvement of the lymph nodes or presence of metastases is not necessarily a contraindication to this procedure. Two women in our series had lung metastases diagnosed preoperatively and neither of them has developed a progression of the disease 10 and 12 years after transplantation.

In our experience the histological features of CH are frequently seen in the late post transplant biopsy samples even if the recipients' liver function tests continue to be within the normal limits. The majority of such cases tends to be attributed in the literature to a viral infection or an autoimmune disease, usually

Table 2 – Histological features in protocol biopsies

Histology	1 year N=5	3 year N=4 (and 1 small marginal sample)	5 year N=4	7 year N=3	10 year N=3
Normal or mild nonspecific changes	1	1	1	0	0
Mild inflammation	4	1	2	1	1
Moderate inflammation	0	2	1	2	2
No fibrosis	4	2	1	0	0
Mild fibrosis (periportal)	1	2	2	2	1
Septal fibrosis	0	0	1	1	2

recurrent, but sometimes acquired "de novo" but many centres have published a number of cases where no obvious cause of their hepatitic reaction could be identified. One possibility, currently debated, is "de novo autoimmune hepatitis" (AIH), which has been documented in the paediatric population and more recently described in the adults [10, 11]. We are aware of the problems in terminology using the word "auto"immune, as the transplanted liver tissue obviously is not an "autotransplant". Nevertheless this is how this terminology has been generally used and it is understood that the causation of this process is related to the presence of antibodies and to the dysregulation of the recipients' immune system. The antigen targets of this liver-specific autoimmunity are species specific, therefore shared by both the recipient's and donor's livers and the graft becomes re-populated by the dendritic cells of the recipient's origin. The term "de novo AIH" with all its clinical and therapeutic implications will probably remain in use until the pathogenesis of this condition is clarified [12]. It is assumed that in the paediatric population de novo AIH is probably related to the effect of immunosuppressive drugs interfering with normal maturation of T-cell [13]. Early diagnosis of this condition is important, because promptly initiated therapy is often successful. Only one of our recipients developed an isolated positive test for antinuclear factor antibody during an episode of severe cellular rejection caused by cessation of the immunosuppressive therapy due to recipient's non-compliance. All patients in our series had histological features of unexplained chronic hepatitis in their protocol biopsy samples without any symptoms or clinical evidence of viral hepatitis or autoimmune disease (recurrent or acquired). As the inflammatory changes were mild and considered to be unexplained allograft hepatitis in the recipients with good liver function and normal liver function tests, the modification of their immunosuppressive therapy was not thought to be necessary. During the past decade the diagnostic criteria of so-called late cellular rejection, which may resemble viral or autoimmune hepatitis, have been discussed [14]. Its histological features, when compared with the morphological features of an early acute rejection, namely the interface inflammatory activity, a lesser degree of bile duct damage and mild subendothelial inflammation also correlate poorly with the clinical and laboratory findings [15, 16].

Conclusion

The reason for the liver transplantation in all of our patients was an inoperable and otherwise incurable epithelioid haemangioendothelioma that is often slowly progressive but has an unpredictable biological behaviour. The treatment by OLT has been successful as shown by the patients long survival, but they all developed chronic, slowly progressive, probably alloantigen dependent hepatitis. Although it is still debatable, whether we should, in similar cases, change our therapeutic approach and modify the immunosuppressive therapy, the follow-up allograft histology seems to suggest that this form of hepatitis is slowly progressive. However, even several years after the transplantation the graft retained its normal function.

References

1. MAKHLOUF H. R., ISHAK K., GOODMAN Z. D.: Epithelioid hemangioendothelioma of the liver. *Cancer* 85: 562–582, 1999.
2. ISHAK K., SESTERHENN I. A., GOODMAN Z. D., RABIN L., STROMEYER F. W.: Epitheloid hemangioendothelioma of the liver: a clinicopathologic and follow-up study of 32 cases. *Hum. Pathol.* 15: 839–852, 1984.
3. MEHRABI A., KASHFI A., SCHEMMER P., SAUER P., ENCKE J., FONOUNI H., FRIESS H., WEITZ J., SCHMIDT J., BÜCHLER M. W., KRAUS T. W.: Surgical treatment of primary hepatic epitheloid hemangioendothelioma. *Transplantation* 80: 109–112, 2005.
4. SYN W. K., NIGHTINGALE P., GUNSON B., HUBSCHER S. G., NEUBERGER J. M.: Natural history of unexplained chronic hepatitis after liver transplantation. *Liver Transpl*. 13: 984–989, 2007.
5. SHAIKH O., DEMETRIS A. J.: Idiopathic posttransplantation hepatitis? *Liver Transpl*. 13: 943–946, 2007.
6. ISHAK K., BAPTISTA A., BIANCHI L., CALLEA F., GROOTE J. D., GUDAT F., DENK H., DESMET V., KORB G., MACSWEEN R. M., PHILLIPS M. J., PORTMANN B. G., POULSEN H., SCHEUER P. J., SCHMID M., THALER H.: Histological grading and staging of chronic hepatitis. *J. Hepatol*. 22: 696–699, 1995.
7. MEHRABI A., KASHFI A., FONOUNI H., SCHEMMER P., SCHMIED B. M., HALLSCHEIDT P., SCHIRMACHER P., WEITZ J., FRIESS H., BUCHLER M. W., SCHMIDT J.: Primary malignant hepatic epitheloid hemangioendothelioma. A comprehensive review of the literature with emphasis on the surgical therapy. *Cancer* 107: 2108–2121, 2006.
8. D'ANNIBALE M., PIOVANELLO P., CARLINI P., DEL NONNO F., SCIARRETTA F., ROSSI M., BERLOCO P., IAPPELLI M., LONARDO M. T., PERRONE R., DONNORSO R.: Epitheloid hemangioendothelioma of the liver:case report and review of the literature. *Transplant. Proc*. 34: 1248–1251, 2002.
9. LERUT J. P., ORLANDO G., SEMPOUX C., CICCARELLI O., VAN BEERS B. E., DANSE E., HORSMANS Y., RAHIER J., ROGGEN F.: Hepatic haemangioendothelioma in adults: excellent outcome following liver transplantation. *Transpl. Int*. 17: 202–207, 2004.
10. HENEGHAN M. A., PORTMANN B. C., NORRIS S. M., WILLIAMS R., MUIESAN P., RELA M., HEATON N. D., O'GRADY J. G.: Graft dysfunction mimicking autoimmune hepatitis following liver transplantation in adults. *Hepathology* 34: 464–470, 2001.
11. MIELI-VERGANI G., VERGANI D.: De novo autoimmune hepatitis after liver transplantation. *J. Hepatol.* 40: 3–7, 2004.
12. VERGANI D., MIELI-VERGANI G.: The impact of autoimmunity on hepatocytes. *Semin. Liver Dis.* 27: 140–151, 2007.
13. CZAJA A. J.: Autoimmune hepatitis after liver transplantation and other lessons of self-intolerance. *Liver Transpl*. 8: 505–513, 2002.
14. DEMETRIS A. J., ADEYI O., BELLAMY C. O., CLOUSTON A., CHARLOTTE F., CZAJA A., DASKAL I., EL-MONAYERI M., FONTES P., FUNG J., GRIDELLI B., GUIDO M., HAGA H., HART J., HONSOVA E., HUBSCHER S., ITOH T., JHALA N., JUNGMANN P., KHETTRY U., LASSMAN C., LIGATO S., LUNZ J. G., MARCOS A., MINERVINI M., MÖLNE J., NALESNIK M., NASSER I., NEIL D., OCHOA E., PAPPO O., RANDHAWA P., REINHOLT F., RUIZ P., SEBAGH M., SPADA M., SONZOGNI A., TSAMANDAS A., WERNERSON A., WU T., YILMAZ F.: Banff Working Group, Liver biopsy interpretation for causes of late liver allograft dysfunction. *Hepatology* 44: 489–501, 2006.
15. BERENGUER M., RAYON J. M., PRIETO M., AGUILERA V., NICOLAS D., ORTIZ V., CARRASCO D., LÓPEZ-ANDUJAR R., MIR J., BERENGUER J.: Are post-transplantation protocol liver biopsies useful in the long term? *Liver Transpl*. 7: 790–796, 2001.
16. HUBSCHER S.: Chronic hepatitis in liver allografts. *Hepatology* 12: 1257–1258, 1990.