CASE REPORT

Liver transplantation recipient with malignant transformation of hepatic adenomas

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ABSTRACT

Hepatic adenomas are uncommon benign liver neoplasms. These tumors can undergo malignant transformation into hepatocellular carcinoma (HCC). Transformations associated with anabolic steroid use are seldom reported. The ability to differentiate hepatic neoplasms is important in pre-liver transplant evaluation. Differentiating hepatic adenomas from HCC has been aided by phenotyping classification systems. We examine the case of a 39-year-old male, former athlete, with β-catenin-activated hepatic adenomas associated with anabolic steroid use that underwent malignant transformation into HCC. Ultrasonography showed an enlarged liver with multiple nodules. MRI images demonstrated multiple tumors with no invasion of biliary tract or major vessels. Biopsies of a segment 5 lesion resulted in a well-differentiated hepatocellular neoplasm (most consistent with hepatic adenoma, β-catenin phenotype negative). Specimens of a segment 7/8 lesion demonstrated a small focus on HCC positive for the β-catenin phenotype. The overall interpretation of the specimens was that the tissue represented early HCC without definite evidence of stromal invasion. Ultimately, an angiogram showed multiple hyper-vascular masses. The patient underwent liver transplantation and has been followed for 130 weeks with no evidence of further tumor progression/recurrence. This experience serves as support towards phenotyping adenomas for β-catenin in order to predict those patients with higher risk of developing HCC. Also, this case supports that adenomas with the β-catenin phenotype have a higher risk of malignant transformation. Such a finding reinforces the concept that transplantation should be recommended for the patient population that is unresectable and who is at higher risk of developing HCC. Additionally, further developments in the techniques of genotyping mutations and deletions will further enhance our ability to stratify risk for HCC transformations.

Key Words: Beta-catenin, Hepatic adenoma, Carcinoma, Hepatocellular, Immunohistochemistry, Anabolic steroids

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is a primary liver cancer. It causes 250,000 - 1,000,000 deaths globally per annum.[1] Risk factors for HCC include: hepatitis B virus (HBV), hepatitis C virus (HCV), hereditary hemochromatosis, cirrhosis, smoking, anabolic steroids, sex hormone replacement therapy, and alcohol abuse.[2] It is usually found incidentally during routine screening examinations. Most recently obesity with resultant hepatic steatosis (NASH) increased risk of HCC.[3]

The evaluation of patients can be difficult. While some patients are asymptomatic, symptoms may include: weight loss, hematochezia, jaundice, fatigue, and ascites.[4] Diagnostic tests such as laboratory tests (liver function tests and AFP), imaging (ultrasound, CT scan, and MRI), and biopsies are used to diagnose HCC.[5] Although the main therapy of

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choice is surgical resection, the majority of patients are not eligible due to the tumor extent or underlying liver condition.[6] Other therapies include transplantation, radiofrequency ablation (RFA), transarterial chemoembolization (TACE), and radioembolization.[7] Post treatment, alpha-fetoprotein (AFP) levels are monitored for surveillance.

Hepatic adenomas are uncommon benign liver tumors.[8] They occur predominantly in women who are taking contraceptives.[9] Less frequently, they are also associated with anabolic steroid use and glycogen storage disease.[10] Hepatic adenomas commonly present with abdominal pain in the right upper quadrant. Diagnostic tests, such as imaging (CT and MRI), are used to diagnose hepatic adenomas. However, the gold standard for diagnosis is pathological evaluation via biopsy.

Surgical resection is recommended for those patients with symptoms due to hepatic adenomas (HA) and those who have large enough lesions.[11] Surgical therapies include: enucleation, resection, and liver transplantation. HA has two common complications: intratumoral bleeding and malignant transformation into HCC. Although rare, the risk of malignant transformation to HCC is 4.2%.[10] After discontinuation of oral contraceptives or anabolic steroids, the tumors may regress in size, however, the risk of transformation remains.[10]

The triggering mechanisms for transformation from HA to HCC are not well understood. Two mutations are associated with hepatic adenomas; TCF1 and β-catenin mutations. TCF1 are more likely to show fatty changes and are less likely to have malignant transformation. On the other hand, β-catenin mutations are less likely to show fatty changes and more likely to show pseudoglandular pattern that has the propensity for malignant transformation into HCC.[1] Unfortunately, there is scant evidence in the literature that supports a definitive mechanism of this phenomenon. In this report, the referenced case supports the use of β-catenin identification as a risk for malignant transformation.

2. CASE PRESENTATION

A 39-year-old male, former athlete (weight lifter) and anabolic steroid user of five years, presented to his primary care physician in June 2012 with headaches and right upper quadrant pain. The patient was 101.9 kg and 177.8 cm with a BMI of 32.23 kg/ml. His past medical history was significant for hypertension and hyperlipidemia. The patient's prescribed medications prior to transplantation included: lisinopril, multivitamins, and inhaled budesonide-formoterol and albuterol.

Laboratory tests and imaging revealed multiple lesions in both hepatic lobes consistent with HA. The lesions were not characteristic of HCC. Thus, arterial enhancement was present, venous washout and microvascular invasion were not present, and the tumors did not have infiltrative characteristics.

He was then referred to our institution. The pre-transplant review of systems was overall non-contributory except for previous anabolic steroid use. The pre-transplant physical examination was normal. Laboratory analysis was significant for: ALT 67 (5 IU/L - 40 IU/L), AST 44 (5 IU/L - 40 IU/L), bilirubin 0.8 (0.0 mg/dl - 1.0 mg/dl), alkaline phosphatase 89 (39 U/L - 117 U/L), creatinine 1.0 (0.5 mg/dl - 1.2 mg/dl) BUN 29 (6 mg/dl - 20 mg/dl) and albumin 3.6 (3.9 g/dl - 4.8 g/dl), glucose 104 (60 mg/dl - 99 mg/dl), HCT 41.9 (43.5%-53.7%), and INR 1.0 with a calculated MELD of 6.

The ultrasound showed an enlarged liver (length 22.4 cm) with multiple nodules scattered throughout both lobes (see Figure 1). The MRI showed multiple tumors too innumerable to count (the largest just under 7 cm) with no invasion of biliary tract or major veins (see Figure 2).

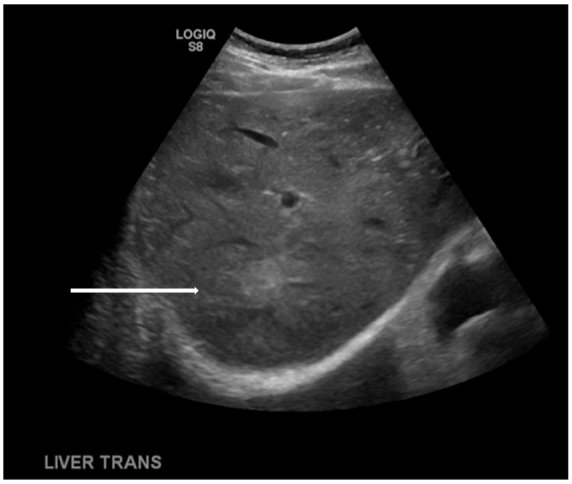

Figure 1. Ultrasound of native liver demonstrating the mass

Pre-transplant biopsy of a segment 5 lesion resulted in a well-differentiated hepatocellular neoplasm (most consistent with HA, β-catenin gene phenotype negative). Specimens of a segment 7/8 lesion demonstrated a small focus of HCC positive for the β-catenin phenotype (see Figure 3). The overall interpretation of the specimens was that the tissue represented early HCC without definite evidence of stromal invasion (see Figure 4). Androgenic therapy needed to be stopped and a key component of the metabolic syndrome (hyperlipidemia potentially leading to hepatic steatosis) controlled in this patient as both appeared to either directly or indirectly increase the risk of neoplastic promotion. Furthermore, β-catenin reactivity in the presence of "multiple" lesions was certainly

of concern for diffuse transformation to frank HCC. The above findings prompted an angiogram which showed multiple hyper-vascular masses in the distribution right hepatic artery, and required subsequent embolization (see Figure 5).

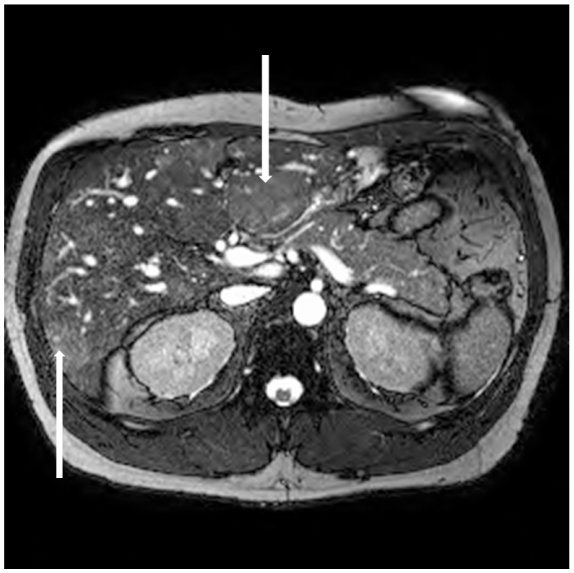

Figure 2. MRI of native liver. Arrows demonstrate multiple masses.

2.1 Intervention

The patient's candidacy for transplant was regionally approved per UNOS guidelines with an exception based on his young age, low probability of spread, and low risk of continued problems of HCC as he was no longer consuming anabolic steroids (as of a year prior to transplant evaluation).

The patient underwent an orthotopic liver transplant, using the "piggyback" surgical technique. The patient had an uneventful length of stay of six days. A follow up ultrasound was performed which showed homogenous parenchyma (see Figure 6). The ultrasound vascular study of the transplanted liver showed normal venous and arterial flow. The gross examination of the recipient liver was positive for well differentiated multiple large tumors (see Figure 7). There were 25 tumors on the right and 16 on the left, with no tumor necrosis present. Lymph nodes were negative for malignancy. The patient was placed on a low dose regimen of maintenance immunosuppressants: 5 mg of tacrolimus daily, 20 mg prednisone, and 500 mg BID of mycophenolate and adjusted accordingly per transplant protocol over the next several weeks.

2.2 Post-operative course

Six weeks post-transplant, liver function tests (LFTs) increased which lead to suspicion of rejection (see Table 1). Biopsy of the liver showed mild acute cellular rejection characterized by portal inflammation including eosinophils and early venulitis (see Figure 8). However, no fibrosis, ductal dilatation, or cholestasis was identified. The patient was treated with methylprednisolone bolus. Two weeks later, the patient demonstrated response to the treatment as shown by a normalization of his LFTs. He was subsequently weaned off prednisone, also his dosages of immunosuppressants were lowered (1,000 mg BID of mycophenolate and 3 mg BID of tacrolimus), and started on 200 mg BID of sorafenib for tumor adjuvant therapy.

At week 16, the patient was hospitalized because of a facial rash, diarrhea, and upset stomach. He had elevated LFTs, which lead to suspicion of rejection (see Table 1). The rejection was confirmed by biopsy as moderate acute on standard H&E sections and was treated once again with steroid boluses. This episode appeared to be more resistant. Mycophenolate, tacrolimus and prednisone were all increased to no avail. The patient finally required thymoglobulin to control this rejection episode. Sorafenib was discontinued, as he did not tolerate it. At 130 weeks after transplant, the patient was free of rejection (stabilization of blood LFTs) and there is no evidence of tumor recurrence or metastatic disease by CT imaging.

3. DISCUSSION

HA malignant transformation into HCC is a rare complication. The exact mechanism of this transformation is not yet understood.[10, 12–15] However, there have been various research groups working to further understand their frequency, risk factors, classification, and treatments. Knowing these factors would allow for better determination of which patients benefit the most from surgical interventions.

In our patient, immunohistochemical methods provided a useful contribution to the data used in determining the candidacy of our patient for liver transplantation. The presence of the β-catenin phenotype concurrent with multiple lesions, were the two most significant findings supporting our decision to move forward with transplantation. As expected, in this setting AFP levels were not useful in helping to determine the presence of significant HCC. The small foci of the β-catenin phenotype on biopsy specimen would suggest that the over-all tumor burden would not have been large enough to provide detectable AFP levels with current standard methods using peripheral blood. As demonstrated by the Larson group, 70% of patients had normal AFP blood levels with hepatic adenomas that were HCC positive as solely identified by DNA analysis of biopsy material.[13]

In 2006, the Bordeaux group genotyped and phenotyped

HAs and determined four tumor subtypes: hepatocyte nuclear factor 1 α-mutated (HFN1α; HA-H), β-catenin activated (HA-B), inflammatory (HA-I), and unclassified tumors (HA-U) (which are tumors without any known genetic abnormalities). HA transformation into HCC was found in 46% of those tumors with the β-catenin mutation. Furthermore, the β-catenin mutation was not found in any of the other subtypes. Thus, those patients with β-catenin mutations are at higher risk of developing HCC.[14,15] This group established and further refined the tool for better characterizing HAs by identifying four immunohistochemical markers that characterize each of the subtypes from the pathomolecular classification. The markers used were liver fatty acid binding protein (L-FABP), glutamine synthase (GLUL), serum amyloid A (SAA), and nuclear β-catenin. When L-FABP was downregulated it indicated HNF1 α-mutation. Nuclear β-catenin staining and glutamine synthase overexpression indicated β-catenin mutation. Finally, SAA positive staining and overexpression of C-reactive protein (CRP) indicated inflammatory HA.[16] These achievements reinforce our choice to use β-catenin as a prognostic indicator for transformation to HCC in patients presenting with HA.

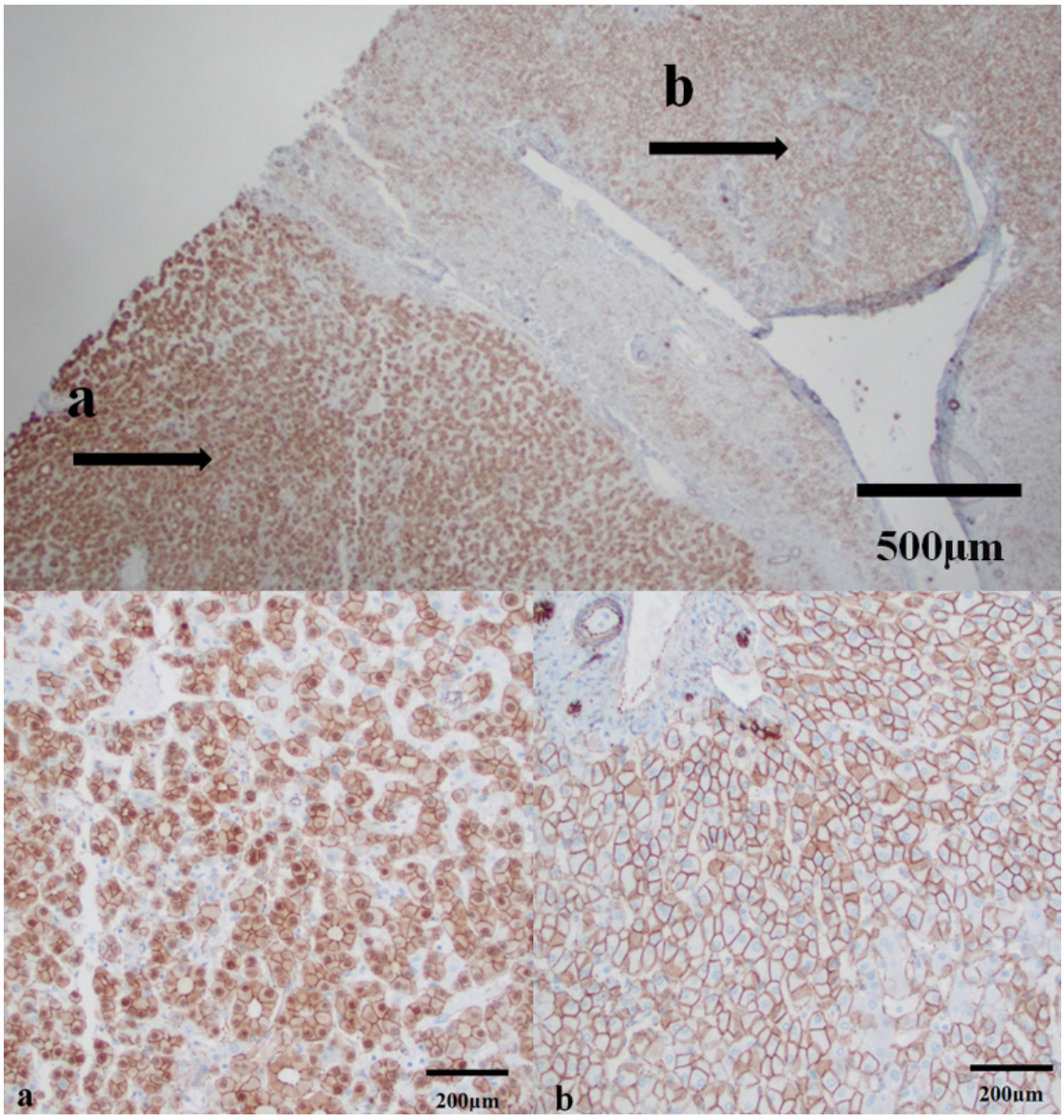

Figure 3. Well differentiated HCC compared to adjacent liver

(a) Immunohistochemical studies for β-catenin demonstrating a positive phenotype (nuclear and cytoplasmic staining) with cell membrane and cytoplasmic staining adjacent to normal liver parenchyma (b) with cell membrane staining only (negative cytoplasmic staining). (Upper: low power 40× (500 μm); Below: high power 100× (200 μm) (β-catenin monoclonal mouse; Cell Marque TM) (Positive and negative controls were appropriate; positive fibromatosis, negative control was the patient sample).

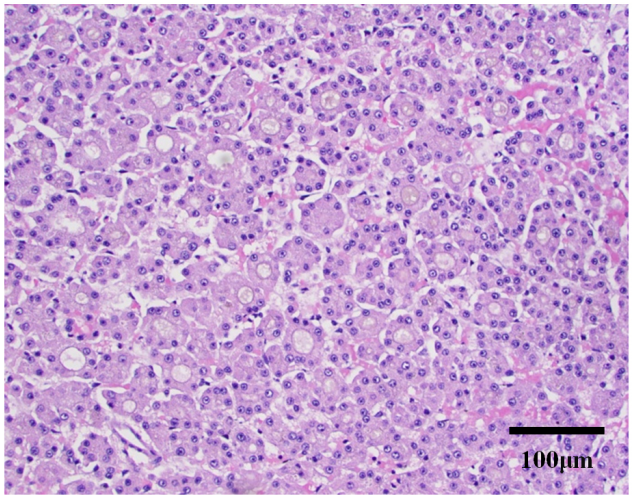

Figure 4. HCC. H&E section showing histologic characteristics consistent with differentiated HCC demonstrating pseudoglandular pattern devoid of portal triads (200×; 100 μm).

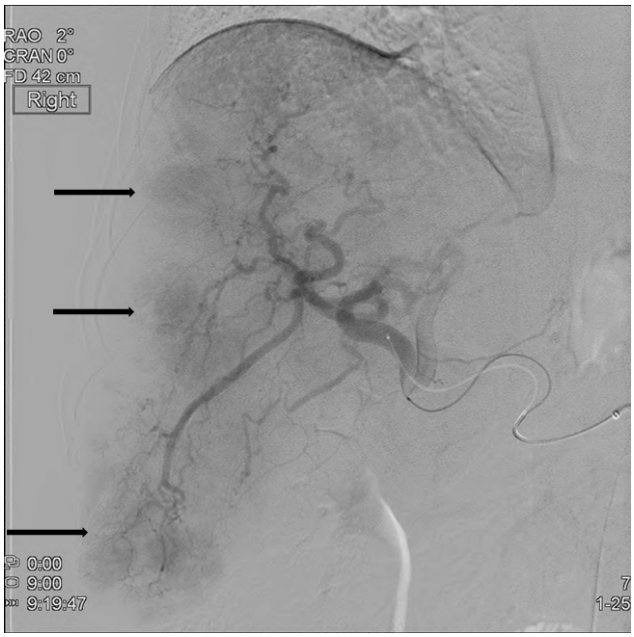

Figure 5. Angiogram of native liver prior to embolization. Arrows demonstrate multiple "tumor blushes".

In 2008, the Hopkins group further emphasized the need for these immunohistochemical findings in HA, that may be useful in predicting HCC transformation. They found, "*cytological atypia without reticulin loss scattered in small foci throughout the background adenomas in two of three cases*" and suggested that atypia may be associated with malignant transformation into HCC. They discussed the findings of the Bordeaux group from 2006. Two mutations are characterized with HA; TCF1 and β-catenin mutations. TCF1 mutations are more likely to show fatty mutations, however, less likely to have malignant transformation. β-catenin mutations are less likely to show fatty mutations, however, more likely to show pseudoglandular pattern and have malignant transformation into HCC.[12, 15]

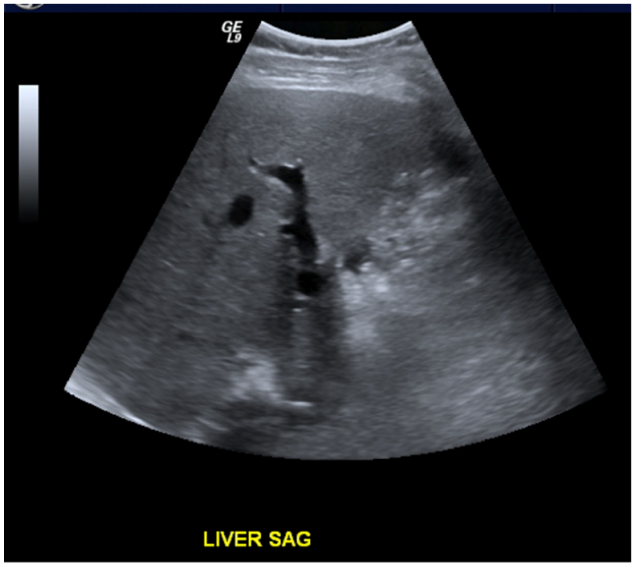

Figure 6. Ultrasound of transplanted liver (without masses)

Figure 7. Gross evaluation of patient native liver. Arrows demonstrate masses.

In 2010, the Maastricht University group suggested that there is a need for a system to identify patients with HA that have higher risk of developing HCC in order to minimize surgical intervention (resection/transplant). They determined the frequency of malignant transformation to be 4.2%.[10] Previous studies have suggested, factors that increase the risk of transformation include: patients with anabolic steroid use, male patients, and patients with glycogen storage disease.[15, 17–19]

Table 1. Patient LFTS on significant dates

LFTS	Pre-OLT	OLT Day	3 Weeks	6 Weeks*	16 Weeks*	130 Weeks
Bili	0.8	2.1	0.6	0.5	6.2	0.4
ALP	89	56	92	83	207	97
ALT	67	911	60	348	1,709	30
AST	44	1,198	32	233	715	20

Note. * Period of rejection

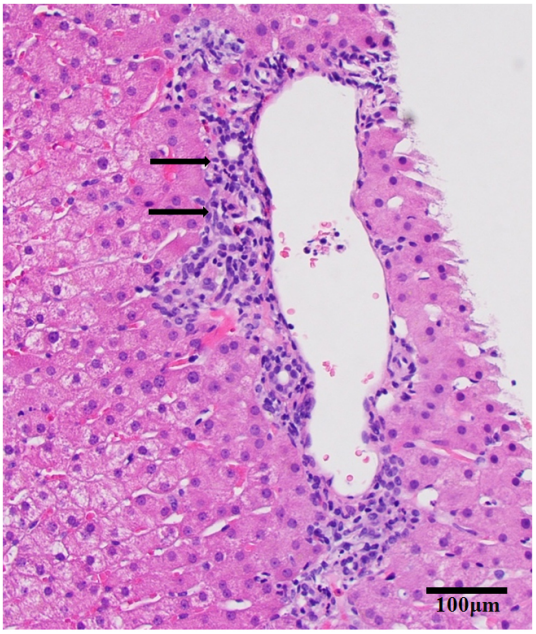

Figure 8. Biopsy of transplanted liver consistent with mild acute cellular rejection (portal inflammation including eosinophils and early venulitis) (100×; 100 μm)

Since the establishment of HA subtypes and markers, management strategies have been under observation. Further research is needed in order to determine and understand the exact mechanism for malignant transformation. In the future, with the use of established risk factors, HA markers and a better understanding of transformation mechanisms, improvements will be seen in the determination of those patients who benefit most from surgical therapies. Further development of genomic/transcriptomic methods (such as PCR, DNA sequencing, RT-PCR, *etc*) will most likely be the cornerstone of improved treatment algorithms. Multidisciplinary teams need to stay up to date with the latest diagnostic tools in order to correctly subtype hepatic adenomas and identify those patients at higher risk for malignant transformation to HCC. Without the aforementioned pathological studies, the utility of the correct surgical approach will be difficult to ascertain.

Conflicts of Interest Disclosure

The authors declare no conflicts of interest.

References

[1] Munoz N, Bosch X. Epidemiology of hepatocellular carcinoma. In: Neoplasms of the Liver, Okuda K, Ishak KG (Eds). Springer. 1987; 3-19. http://dx.doi.org/10.1007/978-4-431-68349-0_1

[2] Davila JA, Morgan RO, Shaib Y, *et al*. Hepatitis C infection and the increasing incidence of hepatocellularcarcinoma: a population-based study. Gastroenterology. 2004; 127(5): 1372-80. PMid: 15521006. http://dx.doi.org/10.1053/j.gastro.2004.07.020

[3] Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and Hepatocellular carcinoma: A weighty connection. Hepatology. 2010; 51(5): 1820-32. PMid: 20432259. http://dx.doi.org/10.1002/hep.23594

[4] Chih-Yi V, Sarna L. Symptom Management in Hepatocellular Carcinoma. Clin J Oncol Nurs. 2008; 12(5): 759-66. PMid: 18842532. http://dx.doi.org/10.1188/08.CJON.759-766

[5] Bialecki ES, Bisceglie AM. Diagnosis of hepatocellular carcinoma. HPB (Oxford). 2005; 7(1): 26-34. PMid: 18333158. http://dx.doi.org/10.1080/13651820410024049

[6] Belghiti J, Kianmanesh R. Surgical treatment of hepatocellular carcinoma. HPB (Oxford). 2005; 7(1): 42-9. PMid: 18333160. http://dx.doi.org/10.1080/13651820410024067

[7] Raza A, Sood GK. Hepatocellular carcinoma review: current treatment, and evidence-based medicine. WJG. 2014; 20(15): 4115-27. PMid: 24764650. http://dx.doi.org/10.3748/wjg.v20.i15.4115

[8] Grazioli L, Federle MP, Brancatelli G, *et al*. Hepatic adenomas: imaging and pathologic findings. Radiographics. 2001; 21(4): 877-92. PMid: 11452062. http://dx.doi.org/10.1148/radiographics.21.4.g01jl04877

[9] Baum JK, Bookstein JJ, Holtz F, *et al*. Possible association between benign hepatomas and oral contraceptives. Lancet. 1973; 2(7835): 926-9. http://dx.doi.org/10.1016/S0140-6736(73)92594-4

[10] Stoot JH, Coelen RJ, De Jong MC, *et al*. Malignant Transformation of Hepatocellular Adenomas into Hepatocellular Carcinomas: A Systematic Review Including More than 1600 Adenoma Cases. HPB (Oxford). 2010; 12(8): 509-22. PMid: 20887318. http://dx.doi.org/10.1111/j.1477-2574.2010.00222.x

[11] Chaib E, Gama-Rodrigues J, Ribeiro MA Jr, *et al*. Hepatic adenoma. Timing for surgery. Hepatogastroenterology. 2007; 54(77): 1382-7. PMid: 17708259.

[12] Micchelli ST, Vivekanandan P, Boitnott JK, *et al*. Malignant transformation of hepatic adenomas. Mod Pathol. 2010; 21: 491-7. PMid: 18246041. http://dx.doi.org/10.1038/modpathol.2008.8

[13] Larson, KA, Weber SM, Fong Y, *et al*. Malignant Transformation of Hepatic Adenoma with Recurrence after Resection. HPB (Oxford). 2002; 4(3): 139-43. PMid: 18332942. http://dx.doi.org/10.1080/136518202760388055

[14] Farges O, Dokmak S. Malignant transformation of liver adenoma: an analysis of the literature. Dig Surg. 2010; 27(1): 32-8. PMid: 20357449. http://dx.doi.org/10.1159/000268405

[15] Zucman-Rossi J, Jeannot E, Van Nhieu JT, *et al*. Genotype-phenotype correlation in hepatocellular adenoma: New classification and relationship with HCC. Hepatology. 2006; 43(3): 515-24. PMid: 16496320. http://dx.doi.org/10.1002/hep.21068

[16] Bioulac-Sage P, Rebouissou S, Thomas C, *et al*. Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry. Hepatology. 2007; 46(3): 740-8. PMid: 17663417. http://dx.doi.org/10.1002/hep.21743

[17] Gorayski P, Thompson CH, Subhash HS, *et al*. Hepatocellular carcinoma associated with recreational anabolic steroid use. Br J Sports Med. 2008; 42: 74-5. PMid: 18178686. http://dx.doi.org/10.1136/bjsm.2007.03932

[18] Franco LM, Krishnamurthy V, Bali D, *et al*. Hepatocellular carcinoma in glycogen storage disease type Ia: a case series. J Inherit Metab Dis. 2005; 28: 153-62. PMid: 15877204. http://dx.doi.org/10.1007/s10545-005-7500-2

[19] Labrune P, Trioche P, Duvaltier I, *et al*. Hepatocellular adenomas in glycogen storage disease type I and III: a series of 43 patients and review of the literature. J Pediatr Gastroenterol Nutr. 1997; 24: 276-79. PMid: 9138172. http://dx.doi.org/10.1097/00005176-199703000-00008