Hepatitis B Surface Antigen as a Marker for Recurrent, Metastatic Hepatocellular Carcinoma After Liver Transplantation

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TO THE EDITORS:

Hepatocellular carcinoma (HCC) is responsible for 695,000 deaths internationally every year and for 19% of liver transplantation (LT) procedures in the United States alone.1 LT for patients with HCC within the Milan criteria has yielded 5-year survival rates >70% and recurrence rates of 13.5% to 17%.2 Controversy remains about surveillance for recurrent HCC after LT. There are currently no standardized guidelines for surveillance because of the relatively low recurrence rates and the absence of demonstrated cost-effective strategies.

The most common etiology of HCC is hepatitis B virus (HBV) infection, which accounts for an incidence rate of 470 HCC cases per 100,000 person-years in North America.3 HBV infiltrates the hepatocyte genome and induces the gradual development of HCC via direct and indirect mechanisms of insult to the genome and hepatocyte growth and differentiation.4 In approximately 11% of cases, HBV may infect liver tumor cells.5 Although this is uncommon, the infection of HCC cells by HBV can lead to positivity for virological markers such as hepatitis B surface antigen (HBsAg) through the integration of the HBV genome into tumor cells. This expression of HBsAg within tumor cells and blood may suggest the development of HCC.6,7 Here we discuss the first published case of recurrent, metastatic HCC after LT that was detected by persistent HBsAg positivity.

A 46-year-old Chinese male with HBV since his late 20s was diagnosed with cirrhosis and HCC in July 2008. The patient was started on lamivudine (100 mg daily) in 2004 and was switched to tenofovir (300 mg daily) in 2009 while he was awaiting LT.

The patient’s initial HCC (a 2.4-cm lesion in segment VIII and a 1.7-cm lesion in segment II) was successfully treated with radiofrequency ablation. The initial alpha-fetoprotein level was normal. There was no evidence of additional hepatic or extrahepatic disease according to imaging performed every 3 months in accordance with United Network for Organ Sharing policy. LT was performed in October 2009. An analysis of the explanted liver revealed 2 lesions that had been extensively affected by radiofrequency ablation and 2 additional lesions (1.8 and 0.7 cm) that had not been noted during pretransplant imaging. All lesions were positive for HBsAg, hepatocyte paraffin 1, and cytokeratin CAM 5.2, and there was no evidence of vascular or biliary invasion (Fig. 1A,B).

For post-LT HBV prophylaxis, the patient received 10,000 U of hepatitis B immune globulin (HBIG) during the anhepatic phase of transplantation and daily infusions of 5000 to 10,000 U on postoperative days 1 to 5 so that he would maintain negative serum HBsAg titers and positive hepatitis B surface antibody titers > 500 IU/mL. Weekly HBIG infusions (5000 U) were then initiated for the first month after LT. The patient was restarted on oral tenofovir (300 mg daily) on postoperative day 1. For immunosuppression, he was treated with a prednisone taper, tacrolimus, and mycophenolate mofetil.

Three weeks after transplantation, the patient’s serum HBsAg results became positive. His hepatitis B surface antibody titers remained >500 IU/mL, and HBV DNA was undetectable by an assay (lower limit of detection = 10 IU/mL). The transient rise in the serum HBsAg level was treated with a switch from tenofovir to emtricitabine-tenofovir (200-300 mg daily) and with the initiation of a daily infusion of HBIG (5000 U) for 3 days and then weekly HBIG infusions (5000 U). His serum HBsAg results transiently became negative, but HBsAg re-emerged 9 days later. His immunosuppression was decreased via the discontinuation of prednisone and the reduction of the mycophenolate mofetil dosage, although these modifications did not result in negative serum HBsAg results. He was given weekly HBIG infusions (10,000 U), which suppressed his serum HBsAg levels for only 24 days (Table 1). HBV genotype and resistance...
profiling could not be performed because serum HBV DNA was undetectable.

The patient underwent abdominal computed tomography 3 months after LT according to our center’s HCC surveillance protocol. The scan revealed a 2.2 cm × 2.9 cm aortocaval lymph node that had increased in size from 1.8 cm. There were no detectable liver lesions. Fine needle aspiration of the node via endoscopic ultrasonography revealed metastatic HCC with features similar to the tumor cells in the explanted liver. Positron emission tomography/computed tomography with [18F]fluorodeoxyglucose demonstrated the presence of a second [18F]fluorodeoxyglucose-avid lymph node within the abdomen. His alpha-fetoprotein level remained normal after LT. In February 2010, the patient underwent laparotomy

Figure 1. (A,B) Explanted liver. The sections demonstrate a well-circumscribed tumor nodule that is morphologically consistent with HCC against a background of HBV-induced cirrhosis [(A) hematoxylin-eosin stain and (B) HBsAg immunostain (×40 magnification)]. (C-F) Excised aortocaval lymph node and wedge resection of a liver allograft. (C,D) The sections demonstrate the complete replacement of nodal tissue by a metastatic carcinoma with a trabecular growth pattern and a focally dilated pseudoacinar formation that is morphologically and immunohistochemically consistent with HCC [(C) hematoxylin-eosin stain and (D) HBsAg immunostain (×200 magnification)]. (E,F) The sections show recurrent HCC with a pseudoacinar pattern that includes prominent bile formation by the tumor [(E) hematoxylin-eosin stain (×200 magnification) and (F) HBsAg immunostain (×400 magnification)].
and retroperitoneal lymph node excision. A pathological examination demonstrated metastatic, well-differentiated HCC in both lymph nodes that was immunoreactive with antibodies directed against HBsAg (Fig. 1C,D). Immediately after the resection, the patient’s serum HBsAg results became negative. The patient was continued on emtricitabine-tenofovir, and he was weaned to monthly HBIG infusions.

In early July 2011, the patient’s serum HBsAg results became positive again (Table 1). Subsequent magnetic resonance imaging demonstrated a 1.1-cm focus in segment VII of the donor liver with a mildly increased signal on T2-weighted imaging that was suggestive of new malignancy foci in the donor liver. The patient underwent wedge resection, and a pathological examination of the resected tissue demonstrated well-circumscribed HCC with a moderately well-differentiated, pseudoacinar morphology that was consistent with both previous liver masses and resected lymph nodes with metastatic disease. The recurrent tumor was immunoreactive for HBsAg and the tumor markers hepatocyte paraffin 1 and cytokeratin CAM 5.2; all adjacent nonneoplastic tissue was negative for HBsAg immunostaining (Fig. 1E,F).

In late February 2012, the patient’s serum HBsAg results became positive again (Table 1). In segments IVa/IVb, V/VI, VI, and VIII of the liver graft, subsequent magnetic resonance imaging demonstrated 4 new foci whose diameter ranged from 0.6 to 1.4 cm. At the time of this writing, transarterial chemoembolization was planned for the patient, and with an inadequate response, the use of sorafenib was possible.

This is a novel case of recurrent, metastatic HCC detected twice via the re-emergence of serum HBsAg. HBV-induced HCC is secondary to a variety of mechanisms.4 HBV integrates into the host’s cellular DNA to disrupt and overpromote genes important to cell growth and differentiation, and it thus causes forcible cell turnover.8 HBV creates viral proteins that directly affect cellular function and favor malignant transformation.9 Additionally, HBV causes the development of HCC by indirect mechanisms of chronic inflammation and cytokine release, which cause fibrosis and hepatocyte proliferation.10

In summary, the persistent presence of HBsAg despite adequate post-LT prophylaxis could be a marker of underlying metastatic or recurrent HCC. In select cases of persistent or recurrent serum HBsAg after LT, we propose its use as a tumor marker for recurrent HCC and the initiation of further investigations with imaging.

| Date       | HBsAg | Hepatitis B Surface Antibody | Viral Load | Treatment/Timing                                                                 |
|------------|-------|-------------------------------|------------|----------------------------------------------------------------------------------|
| 11/19/2009 | +     | +                             | Undetectable | Switch from tenofovir to emtricitabine-tenofovir 5000 U of HBIG daily for 3 days and then 5000 U of HBIG weekly |
| 12/02/2009 | –     | +                             | Undetectable | Discontinuation of prednisone and decrease in mycophenolate mofetil              |
| 01/13/2010 | –     | +                             | Undetectable | Subsequent magnetic resonance imaging of a new 1.1-cm hepatic focus              |
| 02/02/2009 | +     | +                             | Undetectable | 1 day after liver segment VII resection                                          |

In our patient, the tumor itself was infected by HBV. Studies have described this integration of HBV DNA into the host’s genome with specific hybridization.11 HBsAg has also been found in 12% to 15% of tumors from autopsy patients.12 Furthermore, HCC cell lines in vitro express HBsAg because of the HBV DNA integrated into their genomes.13 As HCC tumor cells spread to local lymph nodes and the donor’s hepatic parenchyma, the nodes and the donor liver became new foci for occult HBV, and this leads to persistent or recurrent positive serum HBsAg results.

Positive serum HBsAg results have been found to correlate comparably with direct staining for the presence of HBsAg in tumors.11 Sasaki et al.7 examined predictors of extrahepatic metastasis after hepatic resection in 77 patients with HCC tumors larger than 50 mm. HBsAg positivity was an independent predictor of extrahepatic metastasis, and this indicates that chronic infection with HBV often results in a close correlation between serum HBsAg and HCC even after resection.

In summary, the persistent presence of HBsAg despite adequate post-LT prophylaxis could be a marker of underlying metastatic or recurrent HCC. In select cases of persistent or recurrent serum HBsAg after LT, we propose its use as a tumor marker for recurrent HCC and the initiation of further investigations with imaging.
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