Research Article

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Hepatocellular nodules expressing markers of hepatocellular adenomas in Budd-Chiari syndrome and other rare hepatic vascular disorders

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Background & Aims: A broad range of hepatocellular nodules has been reported in hepatic vascular disorders. It is not clear whether hepatocellular adenoma (HCA) in this context share the same characteristics as conventional HCA. The aim of this study was to carry out a retrospective multicenter survey of hepatocellular nodules associated with hepatic vascular disorders.
Methods: Forty-five cases were reviewed, including 32 Budd-Chiari syndrome (BCS). Benign nodules were subtyped using the HCA immunohistochemical panel.
Results: Nodules with a HCA morphology were observed in 11 cases. Six originated in BCS: two were liver fatty acid binding protein (LFABP) negative (one with malignant transformation); two expressed glutamine synthetase (GS) and nuclear b-catenin, two expressed C reactive protein (CRP). Among three cases with portal vein agenesis, one nodule was LFABP negative, two expressed GS and nuclear b-catenin, both with malignant transformation. In a Fallot tetralogy case, there were multiple LFABP negative nodules with borderline features and in a hepatoportal sclerosis case, the nodule looked like an inflammatory HCA. Two additional cases had nodules expressing CRP, without typical characteristics of inflammatory HCA.
Conclusion: HCA of different immunohistochemical phenotype can develop in hepatic vascular disorders; they may have a different behavior compared to conventional HCA and be more at risk of malignant transformation.

Introduction

Hepatocellular adenomas (HCA) are a heterogeneous group of nodules described usually in normal liver or liver with mild changes and in particular steatosis. HCAs are classified into four major molecular subgroups defined by; (a) mutations inactivating *HNF1A* gene (H-HCA, 35% of HCA); (b) the inflammatory phenotype with mutations of different genes leading to STAT3 activation (IHCA, 50%); (c) the activation of b-catenin pathway by mutations in exon 3 *CTNNB*1 (b-HCA, 15%): among b-HCA, half displayed both inflammatory and b-catenin-activated phenotypes (b-IHCA); and (d) the remaining unclassified tumors (UHCA) [1,2]. HCA is known to occur in association with rare conditions such as glycogen storage disease and familial adenomatous polyposis. Hepatocellular nodular lesions resembling histologically HCA have also been observed in the background of chronic liver disease, in particular hepatic vascular disorders [3–12] and alcoholic cirrhosis [13]. Hepatic vascular disorders are a mixed group of disorders, which include vascular malformative syndromes, e.g. portal vein agenesis, porto-systemic shunts and acquired disorders such as the Budd-Chiari syndrome (BCS). The relationship between hepatic vascular disorders and

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Abbreviations: HCA, hepatocellular adenoma; *HNF1A*, hepatocyte nuclear factor 1A; H-HCA, *HNF1A* mutated HCA; IHCA, inflammatory HCA; b-HCA, b-catenin mutated HCA; b-IHCA, b-catenin mutated inflammatory HCA; UHCA, unclassified HCA; BCS, Budd-Chiari syndrome; FNH, focal nodular hyperplasia; HCC, hepatocellular carcinoma; LFABP, liver fatty acid binding protein; CRP, C reactive protein; GS, glutamine synthetase.

hepatocellular proliferation has long been known. Several types of hepatocellular nodules including focal nodular hyperplasia (FNH), large regenerative nodules/FNH-like, hepatocellular carcinoma (HCC), and nodules resembling HCA have been reported in patients with BCS [3–5]. Unbalanced regional blood flow with arterialization has been proposed as the common mechanism behind hepatocellular proliferation in hepatic vascular disorders [14]. It is the generally accepted pathogenesis of nodular regenerative hyperplasia and FNH, considered to be a relatively non-specific hyperplastic hepatocellular response to aberrant vascular venous perfusion and/or drainage. The observations in hepatic vascular disorders of hepatocellular proliferations within the neoplastic spectrum (HCA, HCC), however, imply that the response to vascular abnormalities is more complex than simple hyperplasia. It certainly constitutes a clinical problem in terms of defining the risk of hepatocellular malignancy for patients with hepatic vascular disorders who develop nodular lesions resembling HCA. In essence, the pathogenesis, biological behavior, prognosis and treatment modalities of a hepatocellular lesion resembling HCA in a patient with hepatic vascular disorders may be considerably different from those of "classical HCA" in a young woman on the oral contraceptive pill and with a normal liver. For this reason we sought to investigate in more detail the immunohistochemical phenotype of these nodular lesions to see how they compare to their more classical HCA counterpart. To our knowledge, perhaps due to their rarity and the rarity of some of the hepatic vascular disorders in which they might arise, a similar systematic study of these lesions has not been carried out to date.

Patients and methods

In this multicenter collaborative study, each participating laboratory contributed with at least one relevant case. Each submitted case had to consist of an example of a hepatic vascular disorder (malformative or acquired) in which hepatocellular nodules had developed, and the histology of which was available in the form of surgical samples. The blocks examined were representative of the whole specimen sampled in each pathological center (livers removed at transplantation, parts of liver removed at surgical resection, or surgical biopsies targeted to nodular lesion at laparotomy). We chose not to include in our study percutaneous lesional liver biopsies in order to minimize sampling error, and to have enough spare tissue to carry out additional staining. Each laboratory was required to contribute to the study by sending the central laboratory histopathologist (PBS) the paraffin wax blocks of formalin-fixed and paraffin embedded representative samples of lesional and non-lesional tissue for each case as well as the original histological diagnosis. Each paraffin wax block was re-cut in the central laboratory. The newly cut sections were used for a set of stainings including Hematoxylin and Eosin (H&E) for initial review by the central pathologist (PBS) followed by a set of immunohistochemical stains for subtyping [15] including the following markers: cytokeratin 7, CD 34, liver fatty acid binding protein (LFABP), C reactive protein (CRP), glutamine synthetase (GS), b-catenin, MIB1, Glypican 3, and Heat Shock Protein 70 (HSP70). The hepatocellular nodules were initially sub-classified into HCC or benign hepatocellular nodule. This latter category included large regenerative nodules (LRN)/FNH-like, FNH as well as HCA. Those nodules with features reminiscent of HCA were further sub-classified into the following sub-categories:

- *H-HCA*: LFABP negative showing typical features namely steatosis.
- *IHCA*: CRP positive showing typical features, namely sinusoidal dilatation, inflammation and aberrant biliary ductular structures.
- *b-HCA*: GS positive (strong and diffuse/patchy) with none of the above pathological features, associated or not with aberrant b-catenin nuclear staining.
- *b-IHCA*: criteria of IHCA in addition to positive immunomarkers of b-HCA.
- *UHCA*: an HCA in which all immunomarkers are negative.
- Those benign nodules showing diffuse expression of CRP, but without any of the other features characteristic of IHCA on H&E were designated as "*CRP positive nodules*". Those nodules resembling large regenerative nodules and with CRP expression restricted to paraseptal lesional hepatocytes, laying along fibrotic inflammatory bands or portal tracts, particularly in cirrhotic liver where few or some or many cirrhotic nodules also expressed CRP on the same section, were not included in this category.
- HCA subtypes with some minor cytological/architectural atypia (thickened cell plates, rosettes/pseudo glands, focal decrease of reticulin), but without obvious characteristics of HCC were classified as *borderline HCA*. These borderline lesions may or may not contain HCC foci.

Age, sex, type, and year of surgery, along with the histological appearance of the background liver, number of nodules and nodule size, as well as some clinical data when available were also recorded.

Results

There were a total of 45 cases, (Table 1) which consisted of 32 cases of BCS, three cases of portal vein agenesis, five cases of hereditary hemorrhagic telangiectasia, three cases of hepatoportal sclerosis, one case of Fallot tetralogy and one case consisting of diffuse hepatic and portal vein obliterative venopathy in a young patient with protein S deficiency and a history of alcohol consumption. In the majority of cases, patients underwent orthotopic liver transplantation and the nodules examined were from the explanted livers. The largest category of liver nodules described in the original histological diagnosis (Table 2) was that of large regenerative nodules/FNH-like (27 cases), which, in three additional cases were associated with concomitant HCC in the liver. HCA was diagnosed in nine cases, three of which showed also concomitant HCC. HCC alone was reported in two cases. In four cases no distinct nodules were identified. The original histological diagnosis of HCA did not include the sub-classification into subtypes in any of the nine cases.

Central pathology review allowed to reclassify some of the nodules (Tables 2 and 3). Of the 27 large regenerative nodules/FNH-like, two were reclassified as HCA (case 8, (b-HCA) and 11 (IHCA)) and two as CRP positive nodules (cases 12 and 13); of the nine HCA originally diagnosed (four in a background of BCS, three in portal vein agenesis, one in Fallot, one in hepatoportal sclerosis), immunohistochemistry allowed to sub-classify them into four H-HCA (LFABP negative), three b-H CA (GS strong and diffuse with or without b-catenin nuclear staining) and two IHCA (CRP positive) (Table 3) (see Figs. 1, 2 and 3).

A total number of 11 HCA was therefore diagnosed after central review and immunohistochemical analysis. They are described in details as follows:

- *H-HCA*. The four H-HCA (two women and two men) occurred in a background of BCS (two cases), portal vein agenesis (one

Table 1. Hepatic vascular disorders studied.

Disease	n	OLT (n)	Surgical resections (n)	Surgical biopsy (n)
BCS	32	28	2	2
PVA	3	1	2	
HHT	5	5		
HPS	3	3		
Fallot	1	1		
HPVOV	1			1

BCS, Budd-Chiari syndrome; PVA, portal vein agenesis; HHT, hereditary hemorrhagic telangiectasia; HPS, hepatoportal sclerosis; HPVOV, hepatic and portal vein obliterative venopathy; OLT orthotopic liver transplantation.