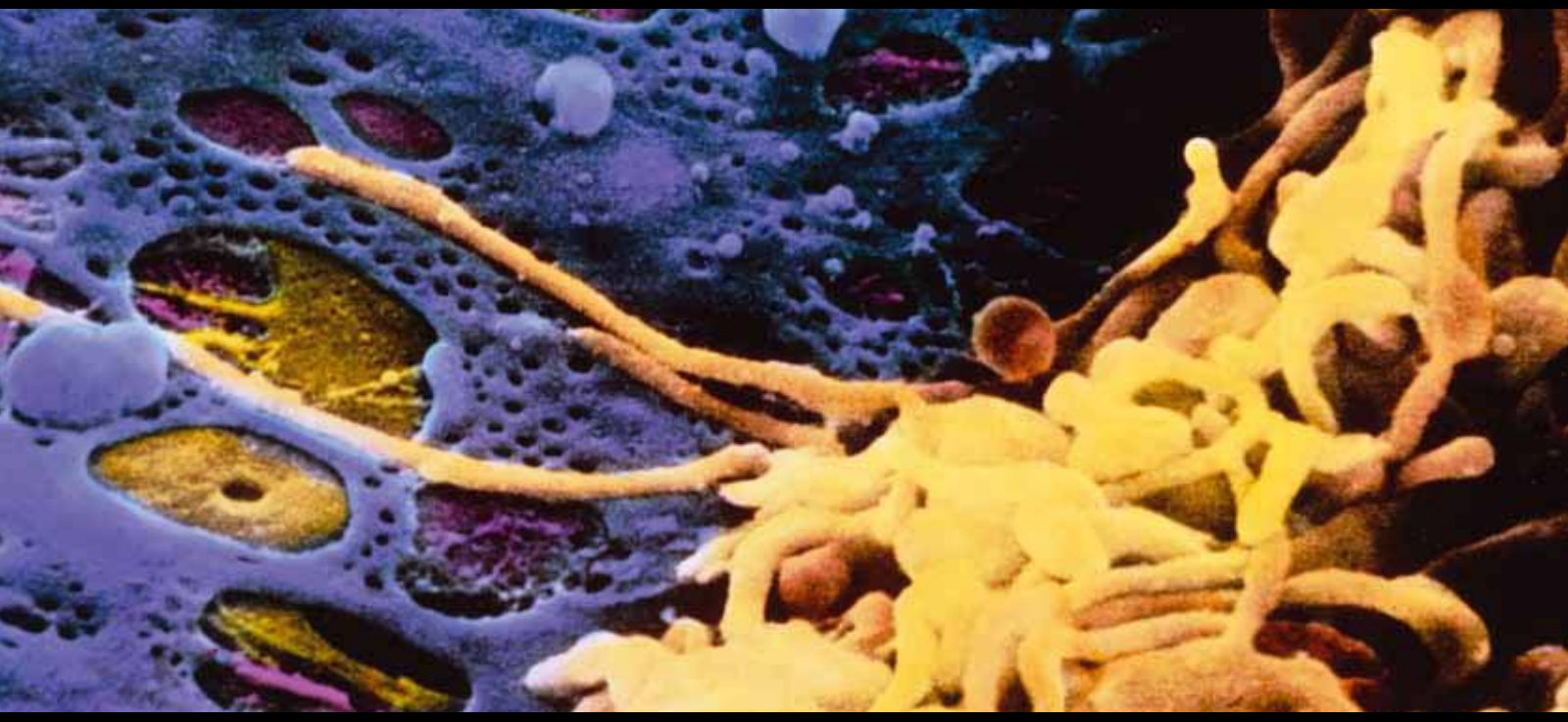
International Journal of Hepatology

Benign Hepatocellular Tumors: A Multidisciplinary Approach

Guest Editors: Paulette Bioulac-Sage, Luigi Grazioli, Türkan Terkivatan, and Charissa Chang



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Editorial Board

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Editorial

Benign Hepatocellular Tumors: A Multidisciplinary Approach

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This special issue is an attempt to cover some new aspects concerning benign hepatocellular tumors: focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA).

In this special issue, we were fortunate enough to have editors from different countries (France, Italy, The Netherlands, and USA) and from different disciplines (liver pathology, liver radiology, liver surgery, and hepatology) who have contributed 6 papers and 5 contributors from academic centres involved in benign hepatocellular tumors, namely, liver pathologists, radiologists, and molecular biologists from different continents: Europe, North America, and Asia.

Concerning FNH, if their diagnosis is mainly made by radiologists, some particular cases required a biopsy or even resection. When standard pathological features are atypical, the diagnosis is greatly facilitated by immunohistochemistry, particularly the characteristic pattern of glutamine synthetase (GS), in biopsy as well as surgical specimens, as described by the Bordeaux group.

HCA is the main topic of this issue. This benign rare liver tumor came to medical attention in 1973 when it became clear that oral contraceptives introduced in the USA in 1960 were the main agents responsible for the occurrence of HCA. The first complication identified was bleeding, which was occasionally life threatening, was often the first manifestation of tumor, and often led to surgical resection. The second complication, hepatocellular carcinoma (HCC) transformation, reinforced a surgical approach to treatment in spite of the fact that malignant transformation remains a subject of controversy.

HCA is a worldwide problem with a clear difference in incidence between Europe and Asia. This difference is mainly explained by the different methods of contraception in the world. In Europe oral contraceptives (OC) are taken by 54, 58, 60, 68, and 75 percent of women in France, Holland, Belgium, Portugal, and Germany, respectively, versus 1% in China, 2% in Japan, and 21% in USA.

In a study from Japan published in this issue, only 2 of 13 cases of HCA described were found in women taking OC. Presently there is no data concerning the influence of the OC generation on the incidence of HCA. It was thought that the lower levels of estradiol should decrease the risk of HCA. This was not observed. The possible explanation comes from the identification of novel risk factors, namely, obesity and metabolic syndrome.

Exploring the combined experience of US centers, publications reporting HCA in the US were identified through a PubMed search and a review of the literature. Whereas earlier reports of HCA in the US described cases exclusively in women exposed to OC, there is a trend towards an increase in HCAs reported in men, HCAs in the absence of OC use, and increased reports of multiple HCAs. This confirms the experience from European centers and may be a result of newer OC formulations and increasing prevalence of obesity.

Interest in HCA has expanded recently in the first decade of this new century when it was discovered that HCAs are different entities under the control of distinct gene mutations. A molecular classification related to risk factors, pathological features, and risk of transformation in HCC was revealed. As reported in the paper describing molecular

classification, three major pathways have been identified which define specific HCA subgroups (1) inactivation of hepatocyte nuclear factor 1A (HNF1A)/transcription factor, (2) activation of the Wnt/b-catenin pathway by *CTNNB1* mutations, or (3) activation of the IL6/STAT3 pathway by somatic mutation of IL6ST, GNAS, or STAT3. The genotype classification led to the phenotypic classification, now well recognized and with specific characteristic immunomarkers. Interestingly, different centres have obtained similar results from the first one published in France. For example in Brussels, as published in this issue, from January 1992 to January 2012, 37 patients underwent surgical resection for HCA. Nine had HNF1α-inactivated HCA (H-HCA: 25%) with lack of LFABP expression; 20 had inflammatory HCA (IHCA: 55.5%) showing CRP and/or SAA expression; in 5 patients (14%), β-catenin-activated HCA (b-HCA) with GS and nuclear β-catenin positivity was diagnosed, two already with HCC. Two cases (5.5%) remained unclassified. One b-HCA exhibited the IHCA histological and immunohistochemical characteristics corresponding to the subgroup of β-catenin-activated/inflammatory HCA. Not surprisingly, as suggested in this issue, the percentage of the different subtypes may differ in HCA not linked to OC use such as in glycogenosis, familial adenomatous polyposis, vascular diseases (Budd-Chiari syndrome, portal vein agenesis), drug intake (danazol), in men and young children. Molecular classification is opening a new field of investigation among these examples of nodules which are not presently well characterized.

In this issue, it was confirmed that MRI was the method of choice to identify HCA (and differentiate from FNH) and that it was possible to identify the 2 major subtypes (H-HCA and IHCA) in the absence of major remodeling due to bleeding/necrosis. A biopsy can be useful when the diagnosis of HCA and HCA subtypes cannot be made.

It was shown that H&E routine histology allows to diagnose >85% of the 2 major HCA subtypes. However, GS is essential to identify b-HCA and b-IHCA. This study demonstrates that a "palliative" diagnostic approach can be proposed, when the panel of specific antibodies is not available. IHCA comprised about 50% of all HCAs and is often associated with obesity as already mentioned. Mild/moderate steatosis is found in the nontumoral liver in a high frequency which is in accordance with the high BMI; of note are the regular findings of sinusoidal dilatation, single arteries, and minute CRP foci which are all features of HCA, as reported by the Groningen's group. These distinct CRP foci are mostly found in cases of multiple IHCA. In academic centers, particularly referral centres for primary liver tumors, immunohistochemistry is essential to understand the natural history of the different HCA subtypes, particularly in women taking OC, and notably with regard to risk for malignant transformation.

In this issue, the Paris pediatric group studied benign hepatic tumors in children. They are very rare. Most FNH remains sporadic, but predisposing factors exist: long-term cancer survivors, portal deprivation in congenital or surgical portosystemic shunting. HCA is frequently associated with predisposing factors such as glycogenosis type I and III, Fanconi anemia (especially if androgen therapy is administered), congenital portal shunt, and SPSS; of note, the same vascular abnormality can induce 2 different types of nodules.

Adenomatosis has been mainly reported in germline mutations of *HNF1-A* gene (associated or not with MODY3). Adolescents or adults with MODY3 should be tested for the presence of HCA. Conversely young diabetic patients with H-HCA should be tested for MODY3.

The Rotterdam group focused its interest on HCA in pregnancy. They come to the important conclusion that pregnancy in women with an HCA up to 5 cm is no longer discouraged in close consultation with the patient, her partner, and members of the liver expert team. In this review you will find also practical guidelines dealing with the diagnosis and management of HCA subtypes in the paper written by authors from different countries.

We hope that his review will encourage multidisciplinary teams to work in this field. We need to establish guidelines (diagnosis, treatment, and follow-up) in different etiological conditions including yet unknown diseases. As an example, the Japanese team found that in patients with alcoholic cirrhosis, a group of possible inflammatory HCAs was characterized by strong immunoreactivity for serum amyloid A (SAA). They suggested that these SAA-positive hepatocellular neoplasms in alcoholic cirrhosis may be a new entity of HCA, which may have the potential for malignant transformation. They correctly indicate that further studies are needed to clarify genetic changes, monoclonality, and pathogenesis of this new type of hepatocellular neoplasm. Understanding malignant transformation of HCA indeed remains a major objective for the coming years.

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Review Article

Pathological Diagnosis of Hepatocellular Cellular Adenoma according to the Clinical Context

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In Europe and North America, hepatocellular adenomas (HCA) occur, classically, in middle-aged woman taking oral contraceptives. Twenty percent of women, however, are not exposed to oral contraceptives; HCA can more rarely occur in men, children, and women over 65 years. HCA have been observed in many pathological conditions such as glycogenosis, familial adenomatous polyposis, MODY3, after male hormone administration, and in vascular diseases. Obesity is frequent particularly in inflammatory HCA. The background liver is often normal, but steatosis is a frequent finding particularly in inflammatory HCA. The diagnosis of HCA is more difficult when the background liver is fibrotic, notably in vascular diseases. HCA can be solitary, or multiple or in great number (adenomatosis). When nodules are multiple, they are usually of the same subtype. HNF1α-inactivated HCA occur almost exclusively in woman. The most important point of the classification is the identification of β-catenin mutated HCA, a strong argument to identify patients at risk of malignant transformation. Some HCA already present criteria indicating malignant transformation. When the whole nodule is a hepatocellular carcinoma, it is extremely difficult to prove that it is the consequence of a former HCA. It is occasionally difficult to identify HCA remodeled by necrosis or hemorrhage.

1. Introduction

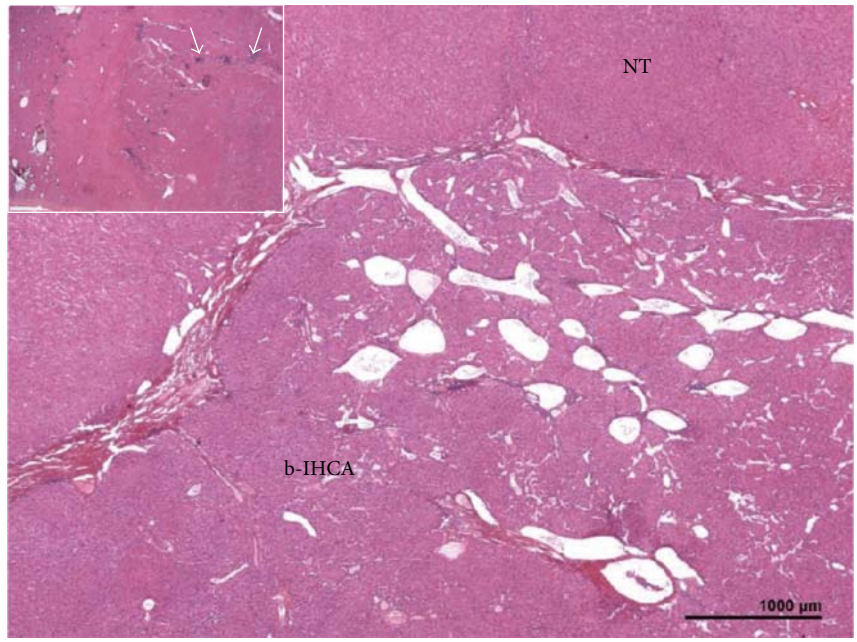
The diagnosis of hepatocellular adenomas (HCA) may occasionally be difficult for the following reasons.

(i) The nodule is discovered in a context different to what we are used to see, such as in men, in women not exposed to oral contraceptives (OC), in older persons, or in children.

(ii) The tumor *per se* may be difficult to identify due to the partial necrosis or to the major remodeling of the tumor leading to the presence of criteria seen mostly in focal nodular hyperplasia (FNH) and/or to difficulties in differentiation from hepatocellular carcinoma (HCC).

(iii) The presence of an underlying liver disease such as nonalcoholic steatohepatitis (NASH), vascular disorder, and fibrosis.

(iv) HCA, HCC, and FNH or different HCA subtypes can be present in the same patient, some more prone



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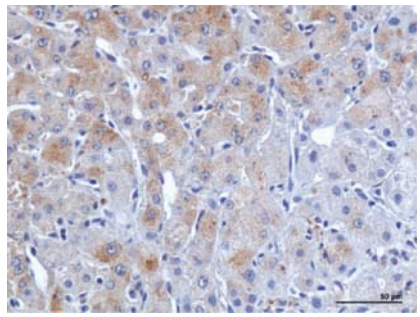

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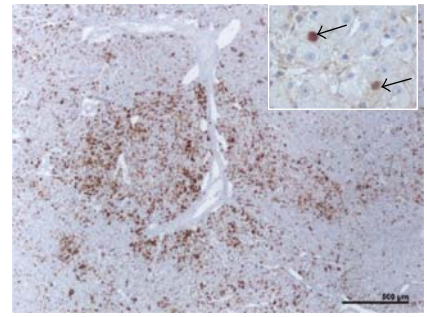


(c)

Figure 1: b-IHCA (with discovery of an HCC 12 years later). A woman born in 1959. Discovery of a nodule 17 cm. Oral contraceptives 4 years, BMI 21.5. Surgery 1984: right hepatectomy but incomplete resection. Followup refused by the patient: 2 pregnancies. Patient seen 12 years later with numerous liver nodules of a well-differentiated HCC. Death occurred few months later. (a) H&E—proliferation of hepatocytes with some atypia (not visible at this magnification) and numerous dilated vessels; no arguments for overt malignancy; inset: a few inflammatory infiltrates (arrows). (b) Moderate expression of SAA by adenomatous hepatocytes. (c) Heterogenous, patchy expression of GS; inset: aberrant expression of a few hepatocytic nuclei (arrows).

to HCC transformation, with the difficult task, in some cases, to differentiate HCA from HCC.

(v) Finally, HCA can be discovered unexpectedly in patients treated for other liver tumors or developed in the context of diseases affecting the liver or other organs.

In this paper, we review the clinical/epidemiological context of HCA, based on our experience (personal cases and consult cases); all these cases being classified according to the pathomolecular classification into four groups, as previously published are HNF1α-inactivated HCA (H-HCA), inflammatory HCA (IHCA), β-catenin activated HCA (b-HCA and b-IHCA) and unclassified HCA (UHCA).

2. HCA: Age, Gender, and Oral Contraceptives

(1) The great majority of HCA occurs in middle age women genitally active, taking OC (at least in many Northern European countries and in North America) [1]. The magnitude of the risk of HCA in OC users is yet defined but is considered to be dose and time dependent. The difference in the incidence of HCA between countries among women taking OC has not been evaluated but seems real. Our experience is presented in Table 1. The incidence seems higher in France than in the US. A possible explanation could be the youngest age of the women exposed to the contraceptive pills in France compared to the US. Less than 20% of women with HCA are not exposed to OC or are exposed for very short periods of time.

Table 1: Hepatocellular adenomas: Bordeaux cases.

	Total	H-HCA	IHCA	b-IHCA	b-HCA	UHCA
n	184*	66	68	13	14	22
Mean age (extreme)	40 (14–66)	41 (14–65)	41 (25–59)	35 (18–59)	35 (14–66)	36.5 (22–52)
n W	163	62	59	8	11	22
Mean age (extreme)	40 (21–66)	41 (23–60)	40 (25–54)	35.5 (26–46)	35 (21–66)	36.5 (22–52)
n W (OC)	146	52	54	7	11	21
n W BMI > 25	52	13	24	0	2	12
n M	19	3**	9	5	2	0

n: number; W: adult women; M: adult men; BMI: body mass index; OC: oral contraceptives; *includes 2 children; **2 patients with MODY3.

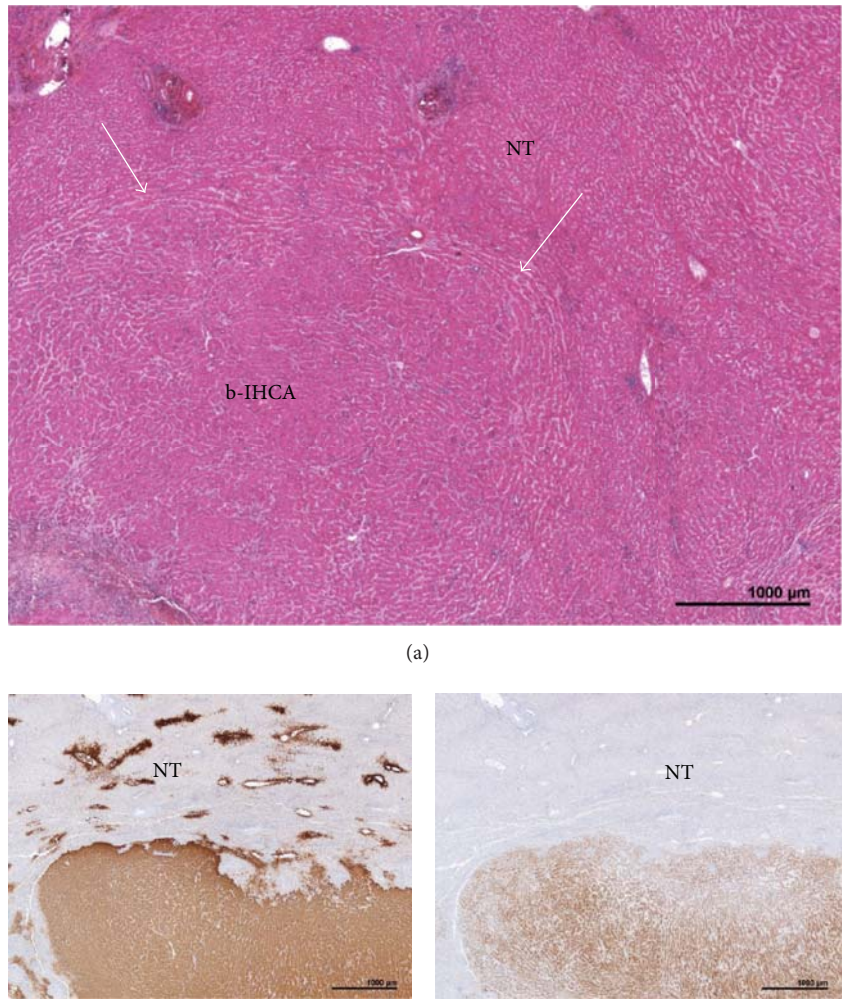
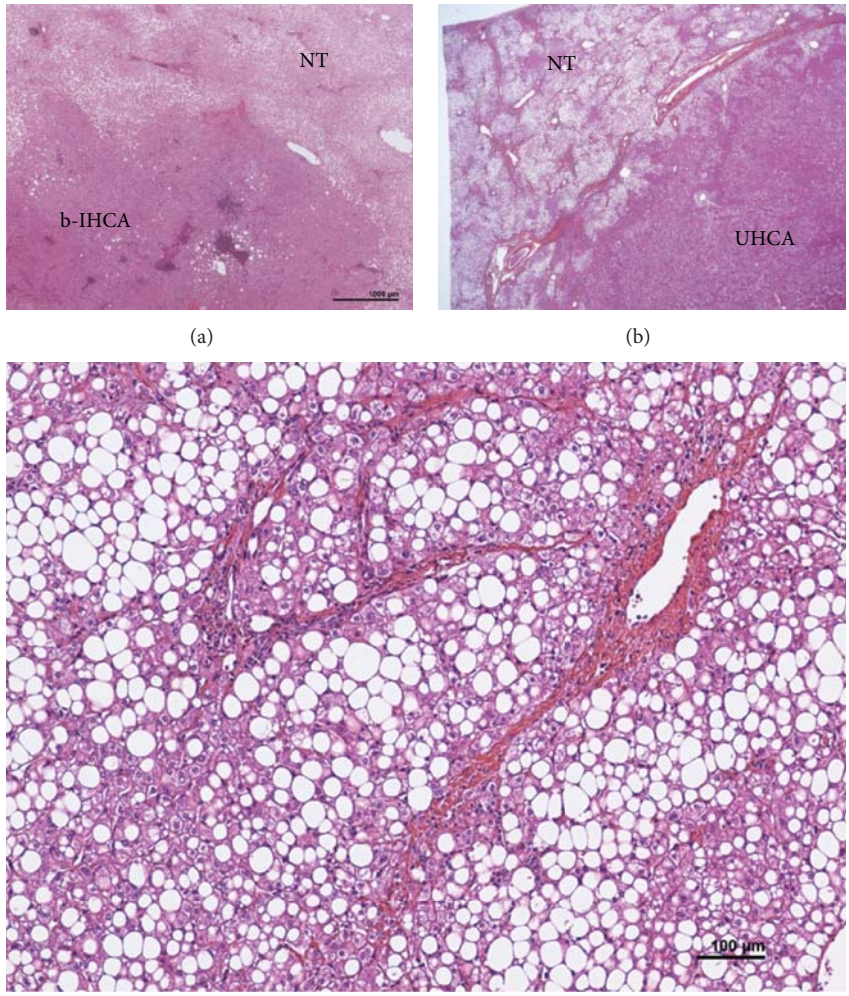


Figure 2: b-IHCA in the context of a FAP. A man born in 1966. Familial adenomatous polyposis (surgery in 1990 and 1996). Liver nodule discovered by chance, 9 cm. BMI 32.3. Segmentectomies V and VI. (a) H&E: HCA with ill-defined border (arrows). (b) Strong and diffuse expression of GS, contrasting with normal GS staining limited to a few centrolobular hepatocytes in the nontumoral liver (NT). (c) Diffuse expression of SAA in adenomatous hepatocytes with sharp demarcation from the surrounding NT.

If the role of OC in the development of HCA is certain, the individual susceptibility to the risk is of paramount importance. No particular HCA subtypes have been observed in women; however, HCA in very young women (in their twenties) taking or not OC without specific etiology are rare and found, in our experience, mainly in the b-HCA and UHCA subgroups.

(2) HCA in men are rare [2–8]. To the exception of HCA cases related to MODY3 (H-HCA) and male hormone administration (b-HCA), the immense majority are found



(a) (b)

(c)

Figure 3: HCA on the background of NASH. (a) A woman born in 1956. Several nodules in the liver, largest 8 cm. Oral contraceptives for 31 years; BMI 24.6. Surgery in 2007 (several tumorectomies and segmentectomies). H&E: IHCA (typical expression of SAA and CRP—not shown), very mild steatosis, contrasting with highly steatotic (60%) nontumoral liver (NT): NASH with mild activity, without fibrosis. (b-c) Woman born in 1973, metabolic syndrome (noninsulin-dependant diabetes, hypercholesterolemia, hypertriglyceridemia). Oral contraceptives 15 years, BMI 31.6. Two liver nodules, largest 26 mm in segment IV. Segmentectomy IVB plus radio frequency of the other nodule (1 cm) in segment VIII. (b) H&E: nonsteatotic HCA (without immunohistochemical characteristics), classified as UHCA; its limit contrast with severe steatotic non tumoral liver (NT). (c) H&E: nontumoral liver: NASH with severe steatosis (80%), mild activity, and septal fibrosis (stage 3).

in the IHCA and b-IHCA subgroups. They are usually solitary. Patients are often overweight with one or several features of the metabolic syndrome. As the risk of HCC is important, resection of the nodule is recommended even for nodules smaller than 5 cm [9, 10].

(3) HCA occurring in infants, adolescents, or young adults are mainly related to specific etiology such as vascular disorders, familial adenomatous polyposis (FAP), or MODY 3.

(4) HCA in patients over 65 are rare. We have observed 4 H-HCA cases, all in women.

(5) In addition to age, gender, OC, and background liver diseases (see below), the biological and radiological parameters are also good indicators of HCA subgroups. Radiologists are now able to identify with confidence typical H-HCA and IHCA. Furthermore, a raised CRP level in the blood is a strong argument to identify IHCA.

3. HCA and HCC

It is well admitted that HCA may transform into HCC [11–25]. However, the risk of malignant transformation of HCA cannot be reliably quantified yet. Several series are concordant to show that approximately 5% of patients, whom HCA has been resected, had pathological evidence of HCC within their HCA. This figure, however, does not take into account fully transformed HCA where evidence of the preexisting benign lesion might have disappeared. The risk

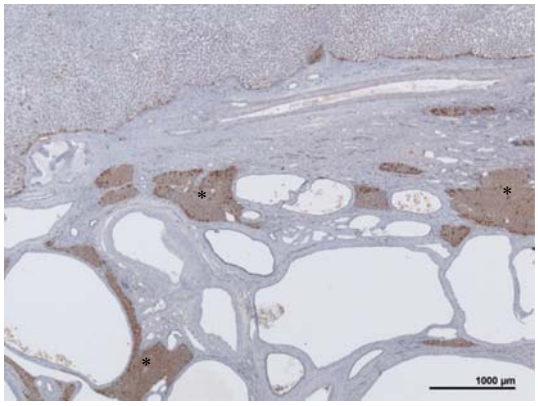


Figure 4: H-HCA associated with polycystic disease. Woman born in 1954. In 1991: kidney transplantation for polycystic kidney disease. In 2000: left hepatectomy for a 11 cm liver nodule. On oral contraceptives for 2 years, BMI 21.2. LFABP immunostaining: numerous biliary cysts with some areas of liver normally expressing LFABP (asterisk), contrasting with a portion of the H-HCA (upper) where LFABP is sharply decreased.

of malignant transformation is correlated with the b-catenin mutated subtype, and with the size of the HCA. HCA malignant transformation is unusual for nodules <5 cm. These results suggest that small HCA occurring in women could be safely observed, as they are also at low risk of bleeding.

HCC that developed on HCA are typically well differentiated without vascular extension or satellite nodules. AFP measurement is not reliable as it is usually normal. The prognosis is—compared to HCC in cirrhotic patients—relatively good if we consider that tumors are usually large tumors (>5 cm).

Prevalence of malignancy within HCA is 10 times more frequent in men than in women and management of HCA should primarily be based on gender. In addition to men, reported cases of HCC on HCA concern rare etiologies such as glycogenosis, male hormone administration, and vascular diseases. Metabolic syndrome also appears as an emerging condition associated with malignant transformation of HCA particularly in men and is the likely most frequent predisposing condition for HCC in this setting. For the pathologists, there are different degrees of difficulties to make the diagnosis of HCA transformation into HCC (see article Balabaud et al. in this issue).

(1) The tumor is definitively an HCC: malignant transformation of an HCA is likely when the HCC occurs in a specific context such as glycogenosis, male hormone administration, or when the diagnosis of HCA has been established several years before. The link exists but cannot be demonstrated when the cause of the putative HCA is not well documented (i.e., in patient exposed to anticonvulsive drugs), or when there are areas in the tumor, particularly at the border, looking benign but that could correspond to a very well-differentiated HCC.

(2) The tumor is benign but there are foci with cytological/architectural criteria in favor of premalignant changes (i.e., rosette formation, increased CD34 staining, irregular/decreased reticulin network).

(3) The tumor is possibly malignant, at least in part (i.e., areas with loss of reticulin, diffuse CD34 staining positivity, GPC3 even mild and focal, etc.). In many occasions, the diagnosis of true malignancy remains impossible to assert and the term "borderline tumor HCA/HCC" can be used. In our own series, we observed 17 cases of HCC possibly linked to HCA with 2 deaths (Figure 1).

4. HCA Occurring in the Context of Specific Etiology

4.1. Vascular Diseases. Many different types of hepatocellular nodules ranging from nodular regenerative hyperplasia (NRH), focal nodular hyperplasia (FNH), and macroregenerative nodule (MRN)/FNH-like, to HCA and HCC have been described in different types of vascular disorders [26–42] such as Budd Chiari syndrome [26–31], hereditary hemorrhagic telangiectasia [32], agenesis of the portal vein, intrahepatic shunts (congenital or acquired) [33–40], and the Fontan procedure [41, 42]. Unfortunately there is today no reliable data using modern techniques of identification of these nodules (imaging, histopathology, molecular biology). Recent data suggest that the majority of nodules are MRN/FNH-like and in addition, different HCA subtypes have been observed with possible malignant transformation [31]. In our personal experience (including consults), we have observed 4 cases (2 with HCC).

4.2. HCA and Genetic Disorders

4.2.1. Glycogenosis. In a large series of 43 patients published in 1997, 51.8% of patients with type 1 and 25% of patients with type 3 glycogen storage disease had HCA at the time of the study. The male to female ratio was 2 to 1 in type 1, and no female had adenomas in type 3 [43]. In a retrospective chart review performed in 117 patients with glycogenosis 1a, it was shown that metabolic control measured on the basis of serum triglyceride concentration may be related to HCA formation [44]. Immunohistochemistry (IHC) has been described in 2 large series of type 1 glycogenosis [45, 46]. IHCA was the main subtype; b-HCA has been also observed but not H-HCA [46]. In our series, we observed in 2 cases different HCA subtypes: IHCA, b-HCA, and b-IHCA. One male patient with type 3 glycogenosis and hepatic nodules detected at the age of 3 died at the age of 27 of HCC in our unit; the diagnosis of HCA and HCC was based on radiological criteria. HCC is a rare but major complication of glycogenosis [17].

4.2.2. Familial Adenomatous Polyposis. HCA in patients with FAP have been reported previously [15, 47–50] with inactivation of HNF1α [48] as well as biallelic inactivation of the APC gene [49, 50]. Malignant transformation has also been described [15]. In our series we have observed 2 cases of b-IHCA associated with FAP (Figure 2).

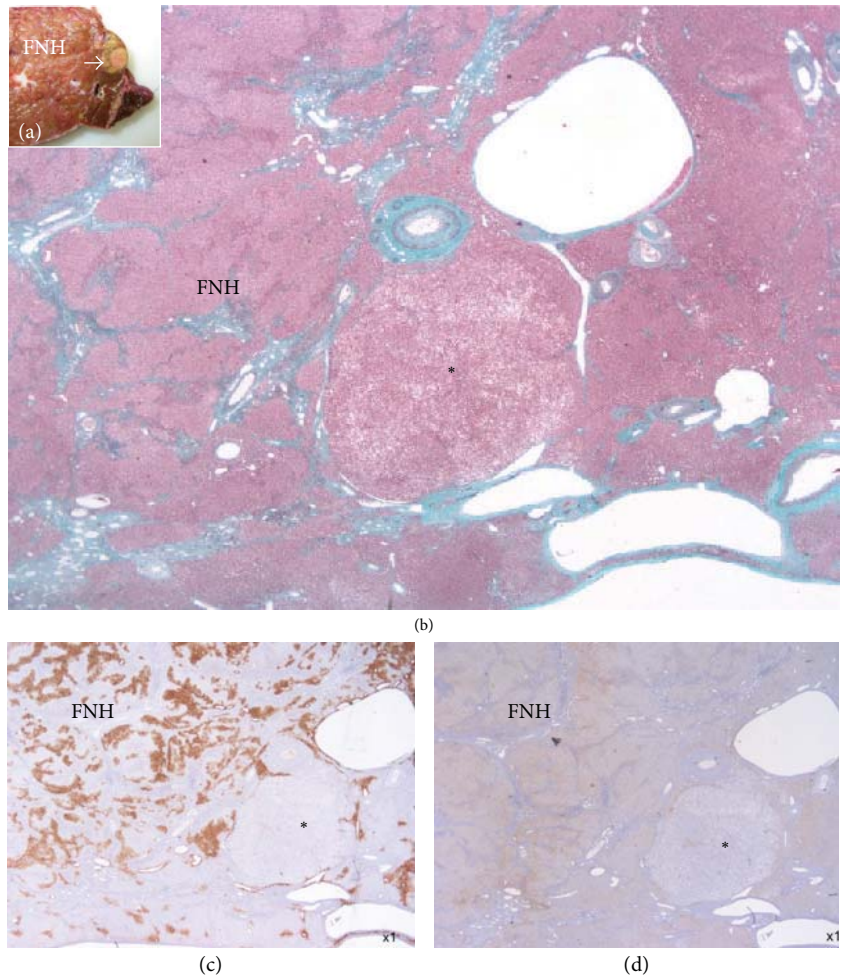


Figure 5: FNH associated with H-HCA. Woman born in 1966. Three FNH, largest 10 cm. Surgery in 2008 (segments V and VI). Discovery on the resected specimen of several small H-HCA. (a) Fresh specimen: typical FNH closed to a small yellow nodule (arrow). (b) Masson's trichrome: typical aspect of FNH, nearby a small steatotic nodule (asterisk). (c) GS staining is negative in the nodule, contrasting with map-like positivity in FNH. (d) LFABP is lacking in the small nodule, whereas it is normally expressed in FNH (as in nontumoral liver, not shown).

4.2.3. MODY3. The discovery of H-HCA in MODY3 is a great success of molecular biology with important clinical consequences [51–55]. The diagnosis of MODY3 should be evoked in H-HCA in the following circumstances: young age of the patient, adenomatosis, history of familial HCA, and diabetes in young age. H-HCA in men are observed only in MODY3 patients. We have confirmed the diagnosis of H-HCA due to MODY3 in 2 families [53, 55].

4.2.4. Tyrosinemia. Cases of HCA have been reported in tyrosinemia [1]. The diagnosis of HCA, in cirrhotic patients, remains very difficult to establish.

4.3. Drugs. HCA and HCC have been reported in patients taking male hormones for medical purposes, that is, Danazol [56–59], or to increase their muscular mass such as body builders [60, 61]. We had at least 2 cases of women taking OC and exposed also to Danazol; both were b-HCA. In one case there were multiple nodules, the largest one shrunk massively after stopping Danazol and all nodules presented features of involute HCC. The link between HCA and the long-term exposition to antiepileptic drugs is not well established [62–66]. We saw 3 such cases possibly linked to antiepileptic drugs.

4.4. Overweight/Obesity. The number of HCA noticeably increased faster in the 2001–2011 period compared to the 1990–2000 period. This phenomenon concurred with an increasing number of patients overweight or obese [67–69]. More overweight patients are found to harbor IHCA than H-HCA. Females still represent the great majority of overweight/obese patients with HCA. Overweight/obese male or female patients constitute a new entity in the IHCA and β-catenin activated IHCA subgroups. Overweight/obesity may soon represent a major risk of malignant transformation of HCA, possibly because of the activation of the IL-6 pathway. In HCA, the nontumoral liver is usually normal

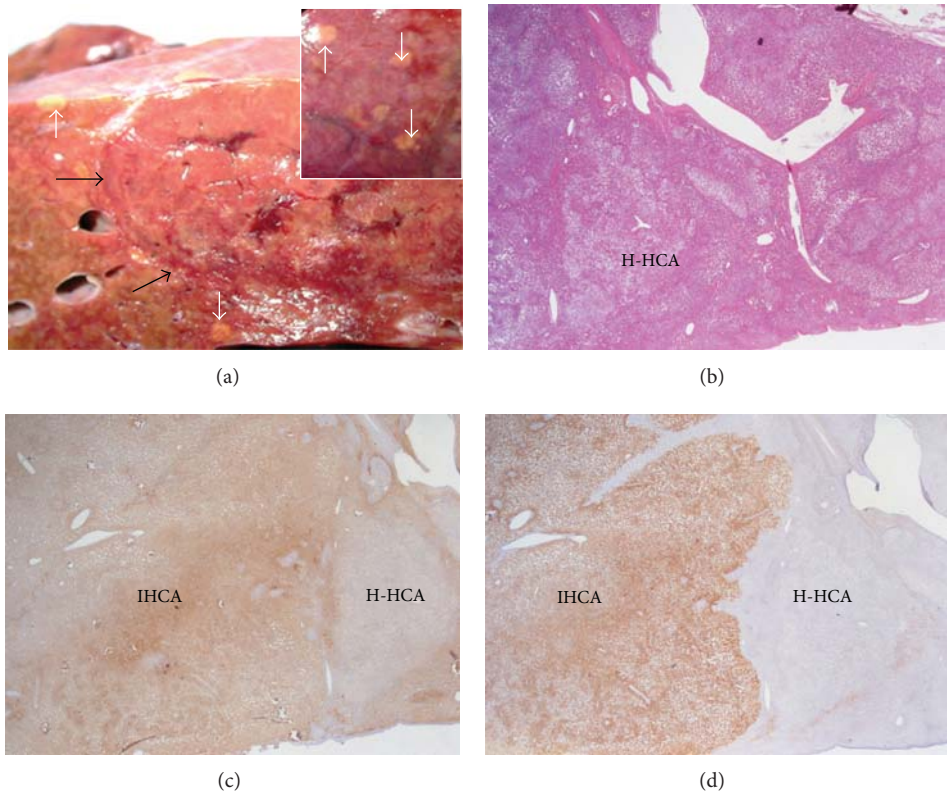


Figure 6: H-HCA and IHCA. Woman born in 1965. Abnormal liver function tests. Hypertriglyceridemia. Multiple nodules largest 9.5 cm. BMI 21.5. 18-year oral contraceptives. Left hepatectomy in 2011. (a) Fresh specimen—the large reddish nodule is not well limited (black arrows), associated with numerous yellowish small nodules in the surrounding resected parenchyma, sometimes visible under the Glisson's capsule (white arrows). (b) The small nodules are steatotic. (c) LFABP is lacking in all the small nodules (H-HCA), contrasting with normal expression in the nearby IHCA. (d) CRP is strongly expressed in IHCA, whereas it is negative in the small nodule of H-HCA.

or subnormal. Steatosis (mild to severe) is quite often observed in overweight/obese patients with or without metabolic syndrome; NASH is rare [70]. Most of the time, there is a clear difference on gross pathology or under the microscope between the tumoral and nontumoral liver (Figure 3). The distinction may be difficult at first glance when the nontumoral liver is steatotic as well as the tumor. Steatosis in the nontumoral liver can also be observed in other instances such as in alcoholic liver disease, glycogenosis, or the Fanconi anemia.

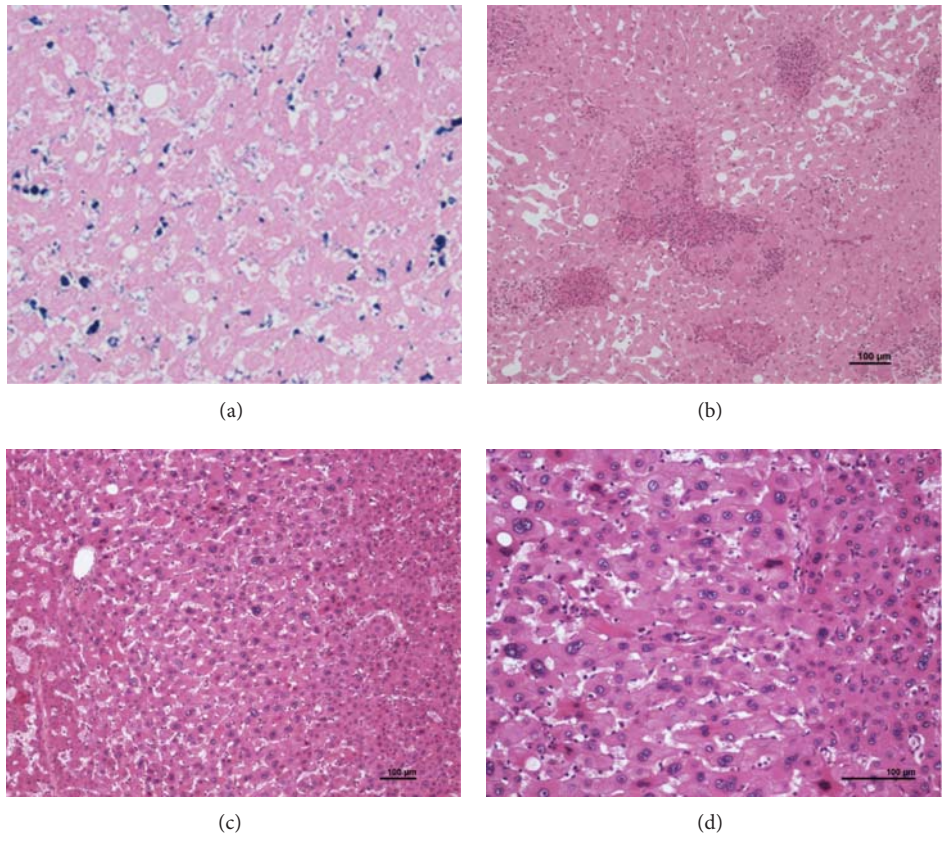
4.5. Anemia. (1) *Fanconi anemia.* The Fanconi anemia is an autosomal recessive disease, causing secondary aplastic anemia and congenital abnormalities, associated with an increased risk of tumors [56, 71, 72]. In patients with the Fanconi anemia, androgen therapy and iron overload may contribute to the development of HCA and HCC; the latter may occur as a transformation of HCA. With prolongation of survival, continued development of liver tumors can be expected. Routine detection should therefore be considered in these patients.

(2) *beta thalassemia*. Hepatocellular adenoma has been reported [1].

(3) *Anemia of chronic disease*. In 2002, severe anemia of chronic disease was described in an unusual group of patients with glycogen storage disease type 1a. The anemia was directly related to the presence of large hepatic adenomas that inappropriately produced hepcidin [73, 74]. A similar mechanism was described in another case not related to glycogenosis [75]. In our experience at least 2 patients were investigated during several years for the diagnosis of inflammatory anemias without any clues until one or several liver nodules were discovered. The anemia was cured after resection of the IHCA [76]. In patients with IHCA, it is not rare to observe biological criteria of inflammatory anemia [10].

4.6. Endocrine Disorders. (1) *Polycystic ovary syndrome.* A case of a young woman with HCA in a context of polycystic ovary syndrome, associated with high levels of androgens and following a high dose hormonal therapy, has been described [77]. We observed a similar case (IHCA).

(2) *Cushing's syndrome.* To our knowledge no case of HCA has been reported yet in Cushing syndrome patients. In our file, one b-HCA occurring in a patient with a Cushing syndrome became malignant years later; however, this old case was poorly documented.



(a) (b)

(c) (d)

Figure 7: Rare, misleading cytological findings. (a) Woman born in 1964. Abnormal liver tests and anemia. No oral contraceptives, BMI 40.4. Alcohol (30 to 40 g per day), hypercholesterolemia, and type 2 diabetes. Biopsy: IHCA. Right hepatectomy 2006: IHCA. The anemia corrected several months after surgery. Perls staining: positivity in sinusoidal cells of the IHCA. (b) Woman born in 1936. Oral contraceptives 21 years. Several liver nodules. Tumorectomies in 1989. H&E: numerous epithelioid granulomas are widespread within the IHCA. (c-d): Woman born in 1947. Massive bleeding. No oral contraceptives, BMI 21.5. Right hepatectomy 2000. Massive liver necrosis. Pathological diagnosis: IHCA. H&E: areas with cytological abnormalities: dystrophic nuclei (irregular, hyperchromatic), nearby necrotic/hemorrhagic zones of the IHCA.

4.7. Fibrotic Background Liver. In severe fibrosis/cirrhosis, the diagnosis of HCA was not reported until recently in alcoholic patients [78]. Here the difficulty is to differentiate an IHCA from a MRN/FNH-like expressing inflammatory proteins. In our experience MRN and MRN-FNH-like, which are frequently observed in cirrhotic background, can express CRP. Molecular data are necessary to identify with certainty HCA in cirrhotic patients.

5. Association of HCA with Other Tumors

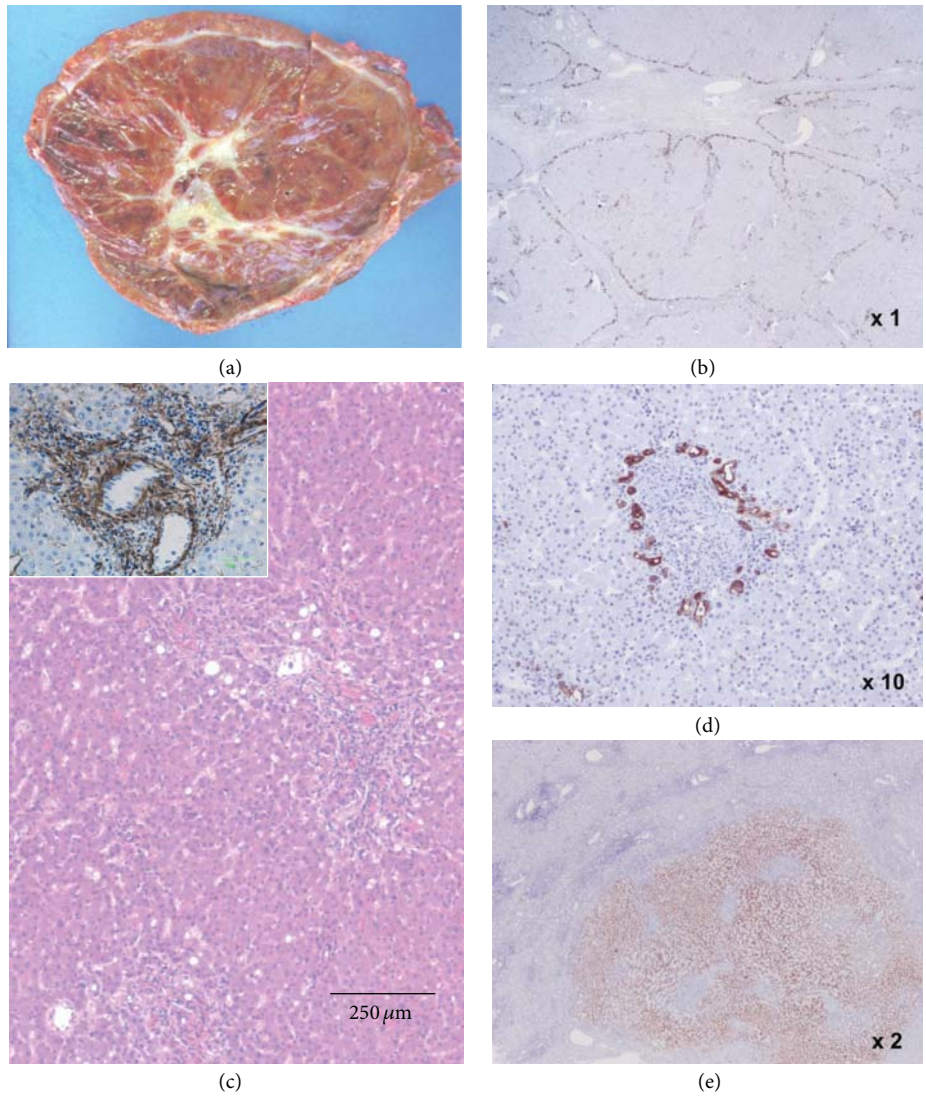
5.1. Association of HCA with HCC and Nonhepatocellular Tumors. We have observed HCA associated with other tumors occurring elsewhere in the liver. In all these cases, HCA were in the H-HCA subgroup, associated with an HCC, an angiomyolipoma, and a mucinous cystadenoma, and one was associated with cysts in the context of a kidney polycystic disease (Figure 4). These associations are possibly fortuitous in the 2 first observations. We cannot, however, rule out the possibility of a common genetic parameter, at least in the last observation.

5.2. Association between Different Benign Hepatocellular Tumors

(i) *Association of HCA and FNH.* The association is probably not fortuitous. FNH are particularly frequent in adenomatosis, and it is not rare that small H-HCA could be fortuitously discovered on the resected specimen (Figure 5). It is well known that liver vascular diseases are prone to the development of FNH. As such they may also play a role in the development of HCA [79].

(ii) *Association of different HCA subtypes.* H-HCA and IHCA are rarely associated in the same liver; we have observed, however, a few cases presenting such association (Figure 6). This association supports the concept of an individual susceptibility to develop HCA, common to several subtypes.

(iii) *Association of IHCA and b-IHCA.* In cases with multiple IHCA, some nodules can be in addition b-catenin activated indicating that b-catenin mutation could be a late event in the development of



(a) (b) (c) (d) (e)

Figure 8: HCA looking like FNH. Same as patient 7A. (a) Fresh specimen: large subcapsular reddish tumor, with a white stellate area of loose fibrosis in the center. (b) CK7 is expressed at the border of fibrotic bands delineating nodules. (c) H&E (αSMA insert). The overall aspect is in favor of an IHCA. (d) Ductular reaction around inflammatory pseudo-portal tracts. (e) CRP is diffusely expressed in the nodule. The diagnosis of IHCA was reinforced by the disappearance of the chronic anemia after resection of the nodule.

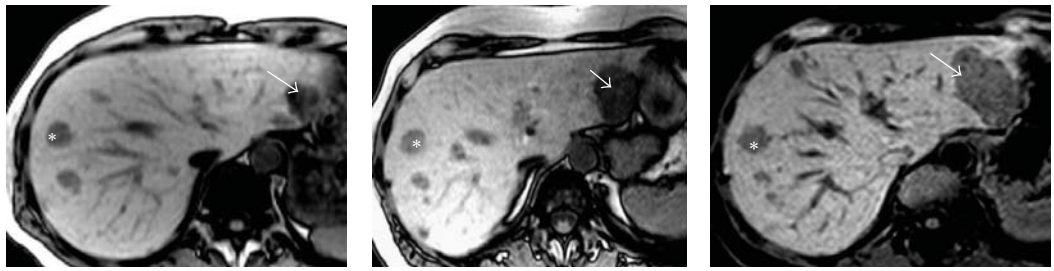
the nodule; indeed, in such cases large nodules were b-IHCA whereas the small ones were IHCA.

5.3. Association of HCA with Extra Hepatic Tumors. (1) In 1989, Wanless et al. reported 13 cases with multiple FNH associated with other lesions such as hemangioma of liver meningioma, astrocytoma, telangiectasia of the brain, berry aneurysm, dysplastic systemic arteries, and portal vein atresia [80]. In the same paper, Wanless described the so-called "telangiectatic subtype of FNH" which occurs in this syndrome as well as in a minority of patients with solitary FNH. Today we know that the so-called telangiectatic FNH are IHCA [81, 82]. FNH with major sinusoidal dilatation, however, does exist [83]. We still have to clarify the association between the above brain tumors and benign hepatocellular nodules.

(2) HCA are so rare that patients with cancer (breast, colon, etc.) and a nodule in the liver are thought to have, *a priori*, a metastasis. We have encountered 3 patients already treated by chemotherapy where HCA were discovered by chance during the followup.

6. HCA with Rare or Misleading Features

6.1. Rare Findings in HCA. Iron [74] or other pigments such as lipofuscin granules, Dubin Johnson pigment [7, 8, 16, 84], hematopoiesis, calcifications [85], and inflammatory granulomas [86–88] are occasionally observed in HCA. Iron is located in hepatocytes in the Fanconi anemia. Surprisingly no iron was observed in HCA with chronic anemia related to hepcidin production. In our experience, among our cases of HCA associated with chronic anemia, we did not observe iron



(a) (b) (c)

Figure 9: H-HCA size increment. Woman born in 1952. Abnormal liver function tests. Oral contraceptives 13 years, BMI 25.4 MRI adenomatosis, largest nodule 6 cm. Segmentectomies IV and VI 2001. Follow-up: increase size of one nodule Segmentectomy III, 2010. (a–c) Phased-opposed T1 weighted MR images in 2001 (a), 2007 (b), and 2010 (c) showing multiple hepatocellular adenomas showing hypointense because of the massive fat component. The nodule located in segment II (arrow) gradually increases in diameter between 2001 and 2010. As the 13 mm nodule located in segment VIII (asterisk), other nodules did not change their size.

overload except in 2 cases where iron was almost exclusively found in Kupffer cells (Figure 7(a)).

6.2. Cytological/Architectural Abnormalities Not Linked to Malignancy Transformation. Abnormalities such as mainly dystrophic nuclei or 2-3 cells thick hepatocytic plates are often seen when HCA exhibits large hemorrhagic/necrotic areas (Figure 7(b)). These misleading features are probably linked to a secondary regenerative process.

6.3. HCA Presenting Features of FNH. HCA can also be remodeled following necrosis/hemorrhage. Fibrotic bands and even scars can form so that HCA look like FNH; these changes are more frequently seen in IHCA subtypes (Figure 8). The arguments to favor one diagnosis over the other rely mainly on clinical, biological, and IHC data; this may not represent, however, strong enough arguments in some difficult cases.

6.4. HCA Number and Size Variation. HCA are solitary or multiple (from few to many). Many HCA defined the so-called entity adenomatosis (arbitrarily more than 10 nodules). For surgeons, adenomatosis is defined also by the number of nodules whose size raises therapeutic decision. Therefore, we have to distinguish cases with a single large nodule accompanied by myriads of small millimetric nodules from cases with multiple large nodules. Adenomatosis is more frequently observed in H-HCA and to a lesser extent in IHCA [10]. Apart from metabolic disorders, adenomatosis is rare in b-HCA and UHCA. Interestingly enough in the immense majority of cases, HCA when detected seems to be already at their maximum size (for those not resected). During followup (and after OC stopped), size remains stable or decrease. The impression, that needs to be confirmed, is that H-HCA remains stable and that IHCA tends to decrease. It is necessary to remember that in HNF1α-inactivated adenomatosis, there are myriads of small HCA that tend to aggregates to form larger nodules [89]. We have observed 2 HNF1α adenomatosis where such large nodules were interpreted as increasing in size, requiring reintervention (Figure 9). It is not known if the hemorrhagic risk is linked to the apparent global size of the nodule or not.

7. Recommendations and Conclusion

If it is tempting to publish rare cases, such as HCA with unusual cytological abnormalities, HCA in rare pathological context (beta thalassemia, tyrosinemia, etc.), HCA associated with rare hepatic or extrahepatic tumors, HCA have to be classified in subgroups with the help of IHC, to understand the pathophysiology of the disease and, in the long term, to better diagnose liver tumors and adapt the management of the patients. Not all HCA can be correctly classified with histological tools only. To prevent this limitation, it is recommended to freeze tissue, in order to perform molecular studies, that are particularly important to understand unusual and unclassified HCA cases. It is hoped that the pathomolecular classification of hepatocellular adenomas developed on normal as well as on fibrotic liver will help us to make progress in this field.

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Research Article

Focal Nodular Hyperplasia and Hepatocellular Adenoma around the World Viewed through the Scope of the Immunopathological Classification

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Focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA) are benign hepatocellular tumors. The risk of bleeding and malignant transformation of HCA are strong arguments to differentiate HCA from FNH. Despite great progress that has been made in the differential radiological diagnosis of the 2 types of nodules, liver biopsy is sometimes necessary to separate the 2 entities. Identification of HCA subtypes using immunohistochemical techniques, namely, *HNF1A*-inactivated HCA (35–40%), inflammatory HCA (IHCA), and beta-catenin-mutated inflammatory HCA (b-IHCA) (50–55%), beta-catenin-activated HCA (5–10%), and unclassified HCA (10%) has greatly improved the diagnostic accuracy of benign hepatocellular nodules. If HCA malignant transformation occurs in all HCA subgroups, the risk is by far the highest in the β-catenin-mutated subgroups (b-HCA, b-IHCA). In the coming decade the management of HCA will be more dependent on the identification of HCA subtypes, particularly for smaller nodules (<5 cm) in terms of imaging, follow-up, and resection.

1. Introduction

The knowledge of benign hepatocellular tumors, that is, focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA), has considerably progressed in the last 10 years, thanks to molecular biology, followed by immunohistochemical applications. Following these advances, new classification is now largely used, first in France, and more recently in other European, American, and East countries.

2. A Brief Overview of Focal Nodular Hyperplasia and Hepatocellular Adenoma

2.1. Focal Nodular Hyperplasia. It is the second most frequent benign liver nodule (after hemangioma), occurring in 0.8% of an adult autopsy population and has been reported in 0.6–3% of the general population. In 80–90% of cases, FNH is discovered in women in their third or fourth decade. In countries (i.e., China) where OC use has been less prevalent, FNH tends to be a lesion of adult men or children of either gender.

FNH is solitary in 2/3 of cases. Most lesions are asymptomatic and are therefore discovered as incidental findings during surgery, autopsy, or imaging procedures for unrelated symptoms. Large lesions can present with abdominal pain or compression of adjacent organs. Reports of hemorrhage or malignant transformation probably do not exist or are exceptional and require confirmation. FNH lesions may regress with age as shown by disappearance of presumed FNH lesions on serial imaging studies.

The background liver is usually normal. FNH is associated with hepatic hemangioma in 20% of cases. Coexistence with HCA is not rare. Associated lesions outside the liver have been reported such as hemihypertrophy, hemangioma of cervix, vascular malformations of brain, and meningioma.

2.1.1. Typical FNH: Morphological Features

(1) Gross Findings. On cut section, classical FNH is a pale, firm mass measuring from a few millimeters to more than 10 centimeters in diameter. The margin is well delimited, and the mass is lobulated and non encapsulated. The lesion is composed of nodules each measuring 2-3 mm, separated by zones of atrophy that give the lesion a multinodular appearance. The lesion characteristically has a central or eccentric stellate fibrous scar with radiating extensions that partially surround some component nodules.

(2) Microscopic Findings. FNH lesions are composed of nodules of benign-appearing hepatocytes arranged in plates not more than 2 cells in thickness. Steatosis may occur, usually focal. The central scar is often edematous or congested and contains one or more large dystrophic vessels, accompanied by numerous small arterioles. The large vessels have irregular fibrous thickening of the intima with focal thinning of the media. The internal elastic lamina is poorly formed and reduplicated. The portal vein is absent. The central fibrous region has radiating branches composed of portal tract-like structures that contain an artery unaccompanied by portal veins or ducts. When fibrous septation is prominent, the appearance may be indistinguishable from cirrhosis, especially in biopsy specimens. A lymphocytic or mixed inflammatory infiltrate is frequent in fibrous regions. At the interface between fibrous regions and nodules, there are often features of cholate stasis including feathery degeneration of hepatocytes, Mallory-Denk bodies, and a ductular reaction that may be highlighted with CK7 and CK19 immunostaining. Sinusoids adjacent to arterial sources are lined by CD34-positive endothelium. Glutamine synthetase is a very useful immunostain, showing a characteristic broadband of expression in hepatocytes often near the hepatic veins.

2.1.2. Atypical FNH. Incomplete or early forms may lack a central scar; they have an incompletion (or absence) of multinodular organization and sometimes exhibit more or less prominent regions of congestion.

2.1.3. Molecular Features. Clonal analysis using the HUMARA test demonstrated the reactive polyclonal nature of liver cells in FNH in 50–100% of the cases [2, 7]. Messenger RNA (mRNA) expression levels of the angiopoietin genes (ANGPT1 and ANGPT2) involved in vessel maturation are altered, with the ANGPT1/ANGPT2 ratio increased compared with normal liver, cirrhosis, and other liver tumors. These data support the importance of vascular alterations in the pathogenesis of FNH. The beta-catenin pathway is activated, including the downstream target, glutamine synthetase [9]. This activation explains the expansion of hepatocytes expressing glutamine synthetase that is so useful for histologic diagnosis. The molecular mechanisms of this activation are uncertain, but do not involve demonstrable mutations in beta-catenin or Axin1.

The pathogenesis of FNH is not fully established. The association with conditions having local or systemic vascular anomalies and the presence of unusually large vessels within the lesions has led to the belief that FNH is a nonspecific response to focally increased blood flow.

2.2. Hepatocellular Adenoma. It is a rare benign liver neoplasm composed of hepatocytes. The incidence is around 3-4/100 000 in Europe and North America and is lower in Asia. 85% of cases occur in young women; HCA is rare in children, men, and the elderly.

The major risk factor for development of HCA is the exposure to estrogenic or androgenic steroids. In young women, 80% have been users of oral contraceptives (OC). The risk increases with the duration and type of OC usage. The prevalence appears to be declining as low-estrogen preparations have become more widely used. The lesions usually decrease in size after stopping OC or after menopause. The clinical presentation of HCA may include abdominal pain, abdominal mass, intraperitoneal hemorrhage, abnormal liver tests, or space occupying lesion found incidentally on an imaging study. HCA can be single or multiple. When 10 or

more adenomas occur, the condition is known as adenomatosis. Clinically significant hemorrhage is observed in 20–25% of cases; the risk is highest when the tumors are larger than 5 cm. Malignant transformation to hepatocellular carcinoma (HCC) is rare, but well documented, occurring in up to 7% of cases as reported from referral centers. The risk of transformation varies with the HCA subtype (see below) and with the clinical association, being higher in patients with glycogenosis or androgenic-anabolic steroid use.

2.2.1. General Morphological Data

(1) Gross Findings. Hepatocellular adenomas are typically large globular tumors with prominent vessels in the overlying hepatic capsule. On cut section, the tumor parenchyma is soft and relatively uniform although areas of congestion, necrosis, hemorrhage, or fibrosis are frequent. The margins of the lesion are ill-defined both grossly and microscopically, with little or no fibrous capsule. Lesions vary in size from microscopic up to 20 cm in diameter. In livers with adenomatosis, there may be hundreds of lesions visible as minute ill-defined nodules visible grossly or only microscopically. HCA may be similar in color and texture to the background liver but are more easily seen when there is lesional steatosis, major congestion and hemorrhage or degenerative changes. The background liver is usually normal, though there may be pallor, fibrosis, or brown pigmentation related respectively to fatty liver disease, glycogen storage disease, iron overload, or other diseases.

(2) Microscopic Findings. HCA is typically composed of benign hepatocytes arranged in regular liver cell plates that are usually one, or at most two, cells in width. A pseudoglandular growth pattern may be seen focally. Tumor hepatocytes have cytoplasm that may be normal, clear (glycogen-rich), steatotic, or contain pigment in lysosomes. Nuclear atypia and mitoses are unusual. The tumor parenchyma is supplied by isolated arteries unaccompanied by bile ducts. Variations in this typical pattern are frequently seen in some of the subtypes, as described below.

2.2.2. Molecular Features.

The reader is referred to the paper by Nault et al. in this issue.

Briefly,

(1) HNF1α-Inactivated HCA (H-HCA). The *HNF1A* gene encodes the hepatocyte nuclear factor 1 (HNF1α), a transcription factor that is involved in hepatocyte differentiation. Bi-allelic inactivating mutations of this gene is found in approximately 35–40% of HCA; 90% of *HNF1A* mutations are somatic; in 10%, they are constitutional (germline). Heterozygous germline mutations in *HNF1A* are responsible for an autosomal dominant form of diabetes MODY3 (maturity onset diabetes of the young type 3). In patients with MODY 3 and HCA, there is an additional somatic mutation of the second allele in the tumor.

(2) Beta-Catenin Activated HCA (b-HCA). An activating β-catenin mutation is found in 10–15% of HCA cases. Glul, a target gene of β-catenin, coding for the protein glutamine synthetase (GS) is also upregulated.

(3) Inflammatory HCA. Inflammatory HCA (IHCA), represent more than half of HCA cases. They are characterized by increased expression of inflammation-associated proteins such as serum amyloid A (SAA) and C-reactive protein (CRP), at both the mRNA and protein levels. 60% of these adenomas harbor mutations in gp130. Mutant gp130 activates STAT3 in the absence of its ligand, which is IL-6. Beta-catenin mutations may coexist with gp130 mutations in 10% of IHCA.

(4) Unclassified HCA. HCA without distinguishing histological features and without known mutations represent less than 10% of all cases.

2.3. Diagnosis of FNH and HCA. An accurate diagnosis can be made using imaging techniques in 90% of cases in experienced centers. Contrast enhanced ultrasonography (CEUS) is the first modality of choice for FNH. MRI is the first modality of choice for HCA. In less than 10% of cases, the differential diagnosis of FNH, HCA, and hepatocellular carcinoma (HCC) cannot be solved by imaging alone. A biopsy interpreted by an experienced liver pathologist can resolve most of these problem cases, with standard and/or immunohistochemical stainings. If the biopsy is not definitive, surgery may be advocated.

The first task is to be certain that the biopsy includes the lesion. Therefore, it is recommended that a biopsy be accompanied by a sample of non-lesional liver.

2.4. Differential Diagnosis. HCA is the most frequent lesion to be distinguished from FNH. The histologic diagnosis of FNH requires two main criteria. The lesion must be composed of benign-appearing hepatocytes and must be supplied by altered portal tracts. Adenomas are supplied by isolated arteries, not portal tracts. A source of difficulty, in this regard, is the presence of CK7/CK19-positive ductular elements in HCA of the inflammatory type. The key feature is that glutamine synthetase expression shows a distinctive map-like distribution adjacent to hepatic veins in FNH while expression is diffusely positive in beta-catenin-activated HCA and mostly negative in other types of HCA (see the following).

Macroregenerative nodule/FNH-like in cirrhotic patients and patients with vascular alterations may be difficult to differentiate from FNH or HCA. These nodules share some similarities with FNH but differ in some ways: the beta-catenin pathway is not activated in cirrhotic FNH-like nodules [9]; the ANGPT1/ANGPT2 ratio is not increased; GS is absent or mildly expressed; inflammatory proteins such as CRP may occasionally be expressed. The presence of focal hepatic vein obstruction and the association with Budd-Chiari syndrome suggest a role of outflow obstruction.

HCC may mimic FNH including focal scarring, arterialized sinusoids, and residual portal tract remnants. Warning signs of malignancy include nuclear pleomorphism, high N/C ratio, wide plates, and mitotic figures in the lesion and often cirrhosis in the background liver.

The differential diagnosis between HCA and well-differentiated HCC remains difficult.

3. HCA/FNH throughout the World

The aims of this study are (1) to make a brief general overview of these 2 entities [2–8]; (2) to report results of a survey through different academic centers in France and throughout the world; (3) to report applications of the molecular/immunohistochemical data and of the new HCA classification in practice through the Bordeaux experience.

At the end of the last century it was thought that HCA will disappear with the use of OC of the third generation. This was not the case. There are several reasons for that: the wider use of modern imaging techniques, the better identification of HCA among hepatocellular tumors, and the emergence of new etiological factors such as obesity [10, 11]. HCA still remains a challenge for clinicians, radiologists, and pathologists. The number of publications is still rising (a pubmed search using hepatocellular adenoma as criterion in 1980–1984, 1990–1994, 2000–2004, and 2005–2009 gave 28, 30, 69, and 87 publications, resp.). Surgery (or any other method to eliminate the HCA) is still necessary to prevent the risk of hemorrhage which can be lethal and the HCC transformation. These risks being absent in FNH, surgery is not recommended; surgery is, however, still performed. There are several reasons for that: surrounding organ compression, compression of liver vessels and biliary tree, pain, and perhaps more importantly doubt about the nature of the tumor. From a brief survey performed in some academic centers (Table 1), as well as from other publications reported in this issue or elsewhere, it is obvious that surgery and biopsies are still performed in France, Europe, and the US for FNH and HCA. The percentage of the different HCA subtypes are in the range previously published (Table 1). The great difference concerns Asia where HCA is extremely rare [12, 13] probably because of other means of contraception than oral contraceptives.

Today the use of the HCA immunohistochemical classification [1, 14–19] is spreading. Several centers have published their own data [20–24].

4. The Diagnosis of HCA Subtypes in Routine Practice: The Bordeaux Experience

The pathological diagnosis of benign liver tumors should take into account the clinical, biological [25], and radiological data [26–35] including etiology.

Main informations are summarized in Box 1. The gross anatomy (nodule and nontumoral liver) remains an essential part of the diagnosis and is essential for the sampling. We recommend to take pictures of the sampled areas and to sample all areas that look different. In the non tumoral liver, it is recommended to sample even tiny areas that look abnormal. To facilitate IHC interpretation, we sample areas at the junction of tumor and nontumoral tissue. The histological features are recorded to classify HCA (Box 2). The IHC data observed in benign liver nodules are summarized in Table 2; to reach a diagnosis, not all IHC techniques are used. Markers are used according to the algorithm in Figure 1.

Briefly *H-HCA* represents a homogeneous group of tumors with lobulated contours, showing typically marked and diffuse steatosis, absence of significant inflammation, or nuclear atypia. FABP1, coding for L-FABP (liver fatty acid binding protein), is a gene positively regulated by *HNF1A*, expressed in normal liver tissue and clearly downregulated in this HCA subtype. By immunohistochemistry, there is a nearly complete absence of LFABP staining, contrasting with the nontumoral surrounding liver which appeared homogeneously stained (even though faintly). Therefore, lack of LFABP expression is a very good diagnostic argument for HNF1-alpha-inactivated HCA, specific of this subtype, since there is a very good concordance between this immunophenotype and *HNF1A* mutations. Furthermore, the downregulation of LFABP may contribute to the fatty phenotype through impaired fatty acid trafficking. HNF1A-mutated HCA occurs almost exclusively in women. Nodules can be solitary or multiple. Most of adenomatosis is *HNF1A* mutated. Constitutional mutations can affect both sexes and can be discovered in children, sometimes as a familial form or associated with MODY 3.

B-HCA. This HCA subtype is often associated with specific conditions (i.e., glycogenosis, male hormone administration) and male gender. The lesions are usually solitary (except in glycogenosis) and have an increased risk of malignant transformation, compared to the other subtypes. Steatosis and inflammation are usually absent. Nuclear atypia and a pseudoglandular growth pattern are frequent in this subtype, so that distinction from well-differentiated HCC may be very difficult. By immunohistochemistry, glutamine synthetase is usually strongly expressed in a diffuse pattern, associated with aberrant cytoplasmic and nuclear expression of beta-catenin. When glutamine synthetase staining is heterogeneous and beta-catenin nonconclusive, molecular biology remains the method of choice for identifying beta-catenin mutation.

IHCA. Most patients with IHCA are women. Obesity and fatty liver diseases are frequent. In 50% of cases there are elevated serum levels of CRP and increased erythrocyte sedimentation rate, rarely in association with fever and anemia, features which can regress after HCA resection. Nodules can be solitary or multiple. Micronodules can also be detected by SAA and CRP immunostaining in the liver parenchyma, outside the main tumors. Histologically, IHCA typically exhibits focal or diffuse inflammation, sinusoidal dilatation, congestion and peliotic areas, and numerous and thick-walled arteries often associated with ductular reaction, lying in small amount of connective tissues. Steatosis may be seen, mainly focally. Immunohistochemistry demonstrates strong expression of SAA and CRP restricted to tumoral hepatocytes. There is a risk of malignant transformation, particularly for IHCA which is also beta-catenin mutated.

Reasons for classifying HCA are summarized in Box 3.

Major advances brought by the classification are (a) individualization of FNH from HCA and FNH from FNH-like, (b) identification of MODY3 and patients at high

Table 1: Diagnosis of FNH and HCA performed in different academic centers.

	HCA		FNH		No final diagnosis	
	Surgery	Biopsy	Surgery	Biopsy	Surgery	Biopsy
			French (from 2008 to 2011)			
(1) Besançon	4 (4♀)	10	13	3	0	0
(2) Bordeaux	49 (44♀)	16	27	29	0	2
(3) Caen	22 (20♀)	6	19	6	0	0
(4) Créteil	14 (13♀) 2 with HCC	19 3 with HCC	19	13	0	0
(5) Grenoble	19 (18♀)	18	3	12	0	1
(6) Lille	31 (26♀) 1 with HCC	30	25	39	2	6
(7) Lyon (1)	49 (41♀) 4 with HCC	13	17	16	4	7 (1 with HCC)
(8) Lyon (2)	16 (13♀) 1 with HCC	25	14	22	0	4
(9) Montpellier	32 (31♀) 1 with HCC	28	15	25	3	1
(10) Nice	32 (31♀) 1 with HCC	28	3	25	3	1
(11) Paris (St. Antoine)	11 (9♀) 1 with HCC	13	20	14	0	2 (1 with HCC)
(12) Villejuif (Gustave Roussy)	1 (1♀)	3	5 (4♀)	6	0	4
			International			
(1) Baltimore (from 1984 to 2012)	63 (61♀) 7 with HCC	6	79	54	4	8
(2) Brussels (Cliniques universitaires Saint-Luc-UCL) (1992–2012)*	37 (33♀): 21 IHCA, 10 H-HCA, 1 with IHCA + H-HCA, 3 β-HCA (2 with HCC), 2 UHCA	14	22	14		
(3) Heidelberg (2007–2011)	11 (11♀): 9 IHCA, 1 H-HCA, 1 with IHCA + H-HCA		34			
(4) London Kings (1998–2011)	35 (30♀): 18 IHCA, 7 H-HCA, 1 β-HCA, 9 UHCA					
(5) NY (Mt Sinai) (2007–2011)*	27: 9 H-HCA, 11 I-HCA, 7 UHCA		15			
(6) San Francisco (selected cases)	12 (10♀): 2 IHCA, 3 H-HCA (1 with HCC, 1 borderline), 3 β-HCA (2 with HCC, 1 borderline), 4 UHCA (1 with HCC)					
(7) Seattle (2008–2011)	9 (7♀): 3 IHCA, 3 H-HCA, 1 β-HCA, 2 UHCA		1		1	
(8) Seoul (2008–2011)	2 (1♀): 1 β-IHCA, 1 β-HCA (1 with HCC)		4 (3♀)			
(9) Singapore (selected cases)	2 (2♀): 1 β-IHCA (with HCC), 1 H-HCA					
(10) Taiwan	12 (5♀): 3 IHCA, 2 β-HCA, 1 H-HCA, 6 UHCA					

*See papers in this issue for additional information.

Age, sex
Mode of discovery: emergency, pain, behavior after stopping (or not) OC
Number of nodules, max size, location
Radiological diagnosis: FNH, HCA, HCC, MRN, cannot differentiate
Woman: oral contraception (age beginning, stop), duration, type
    Number of children (age)
Drugs including all types of hormones particularly male, antiepileptic
Habit: alcohol, tobacco
Diseases
    BMI, metabolic syndrome, NASH
    Diabetes, MODY3 (family history)
    Glycogenosis, deficit OTC
    Mc Cune Albright
    FAP
    Vascular diseases
        Congenital malformations, BCS, HPS,
    Biliary diseases: CHF, polycystic kidney diseases
    POCS
    Brain tumor
Family history of liver tumors
Pathological diagnosis
    Biopsy, surgical specimen (safety margin)
    Biopsy: quality
    HCA: H-HCA, IHCA, β-HCA, β-IHCA, UHCA
    HCC
    HCA borderline lesion; HCA with dysplasia, HCC foci
    FNH
    FNH/UHCA?
    FNH
    MRN/FNH-like
    Association of different types of nodules
    Nontumoral liver
Normal; steatosis, NASH, glycogenosis, vascular liver diseases, biliary disease and so forth

Box 1: Clinical and pathological information useful to manage the patient.

risk of malignant transformation, (c) demonstration that adenomatosis was not per se a specific HCA subtype, but an entity defined by an arbitrary number of nodules > ten detected by imaging techniques (the high number linked probably to a specific susceptibility), and (d) correction of errors (the so-called telangiectatic FNH being IHCA).

Practical guidelines for the diagnosis of HCA subtypes are summarized in Figure 2.

The HCA classification has changed the way we consider HCA and FNH in our center. The diagnosis accuracy of FNH and HCA has completely changed at the end of the first 2000 decade. There are probably in the literature many unavoidable diagnostic errors concerning benign hepatocellular nodules particularly in the field of hemorrhagic FNH, malignant transformation of FNH, identification of difficult nodules, and so forth.

4.1. Practical Guidelines for the Management of HCA Subtypes [25, 36–56]. For practical reasons once the diagnosis of liver tumors is made, the patient is often referred to a surgeon. In spite of the vast literature in various domains: imaging, pathology, surgery, complications, and so forth, there is no guidelines for the diagnosis and treatment of HCA. Nevertheless the major contribution of some European centers (see Supplemental Table 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2013/268625) is a good starting point to obtain valuable information.

There is a global consensus among liver surgeons that adenoma >5 cm should be resected if they have not regressed after stopping oral contraceptives. Some surgeons prefer that all HCA should be removed particularly if they are easily accessible laparoscopicaly. Indeed, the clinical presentation in terms of age, sex, oral contraceptives and other major etiology, number of nodules (from single, to several and up to myriads) and size (2 to 20 cm), mode of discovery, and so forth, therefore it is extremely difficult to define acceptable guidelines based on solid arguments. Most of the series published are very small and it is difficult to make a judgment on case report.

This is the reason why we believe that to make progress we need to collect prospective data from different countries and continents. The identification of subtypes is one of the key factors among others that need to be collected (Box 1). The ideal situation would be to follow strictly well- identified (MRI/biopsy) HCA particularly those at a high risk of malignant transformation (β-HCA and β-IHCA).

Gross anatomy: location, size, color, hemorrhage/necrosis, soft/hard
First step: H&E, trichrome, CK7, CD34
Areas of necrosis, hemorrhage, congestion, peliosis
Fibrosis (bands): constitutional versus remodeling, scar, ductular reaction
Steatosis (distribution), sinusoidal dilatation (degree and location), arteries/thickness of the wall/pseudoportal tracts/inflammation
Cytological abnormalities
Nontumoral liver: steatosis, underlying liver disease, micronodules, etc.
Second step (see Figure 1): GS then (if necessary) other IHC markers
At the end should be able to:
Differentiate FNH from HCA
(i) FNH: typical, typical (GS) but lacking key features or with unusual findings (massive steatosis, sinusoidal dilation, involution)
(ii) MRN/FNH-like (cirrhosis, vascular disorders)
(iii) HCA
Subtype HCA
Identify premalignant/malignant HCA lesion (focal or spread)
H&E: rosettes, small cells
Reticulin: disarray, loss
GS: abnormal staining: strong/mild/weak, diffuse/focal/few cells
CD34: normal pattern/diffuse
β-cat.: nuclear staining (many, some, rare)
Additional markers: GPC3, HSP70, clathrin, etc. may be useful
HCA with extensive necrosis/hemorrhage or remodeling may not be identified with certainty
When the diagnosis (FNH versus HCA and HCA subtypes) is not clear on regular stainings and IHC, molecular biology is the next step (techniques on paraffin sections should become available in the near future: this is particularly important concerning β-catenin-mutated HCA)

Box 2: Pathological record.

Clinical research
To investigate the natural history of subtypes (relevant for the follow-up)
To identify MODY patients (genetic counseling)
To convince patients with IHCA in the context of overweight/obesity/metabolic syndrome to be medically followed
To discover nodules with a high risk of malignancy
To find correlation between etiology and subtype
To retrospectively correct diagnostic errors (FNH versus HCA; in cirrhosis FNH versus MRN/FNH-like)
Translational research
Correlation between IHC markers and mutations
New IHC markers to identify new mutations
Research tool
Identification of new mutations (UHCA)
Prognostic factors among patients at risk for malignant transformation according to subtypes
Use the terms of the IHC classification to avoid misnomers (telangiectatic HCA, steatotic HCA, mixed form FNH/HCA, etc.)

Box 3: Reasons to classify hepatocellular nodules using IHC methods.

In the absence of accepted guidelines, our proposition for the management of HCA subtypes <5 cm is summarized in Figure 3.

4.2. Future Developments. It is clear that we have entered a new era in the study of benign hepatocellular tumors and perhaps more importantly in the field of HCC developing in nonfibrotic liver. One may still consider that this breach has not yet dramatically changed our strategies—diagnostic and therapeutic [38]. This is only partly true (see above) because it will take time before the classification brings its full potential. It is true that our knowledge is still limited. Future development will be based on imaging techniques [35], molecular data including chromosomal abnormalities [57], on the ability to combine molecular, radiological, and clinical data, and first of all on the reinterpretation of the

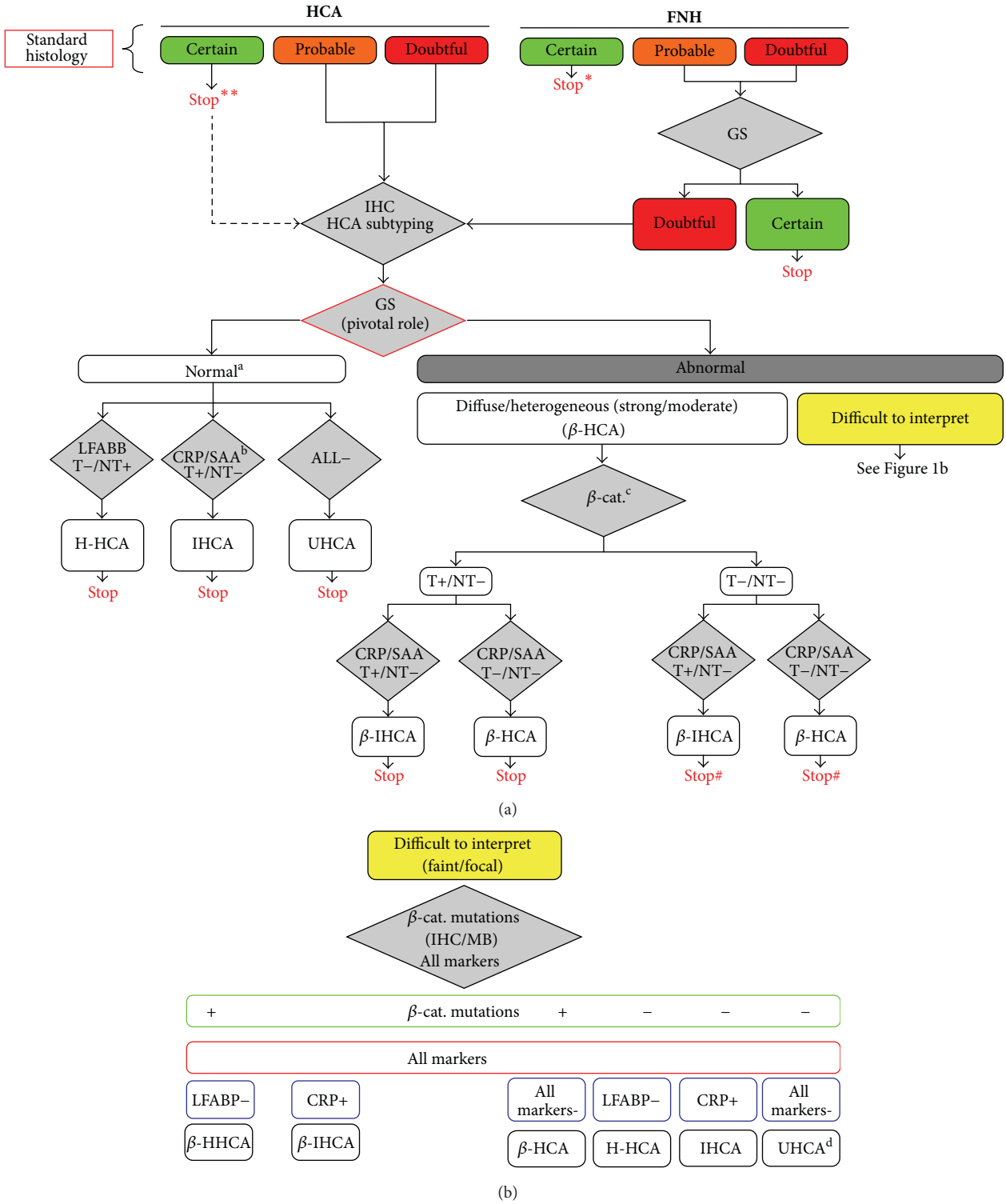


Figure 1: Adapted from Bioulac-Sage et al., [1]. Algorithm for immunohistochemical (IHC) diagnosis of benign hepatocellular nodules: focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA). Glutamine synthetase (GS) is not always mandatory for the diagnosis of *FNH or **HCA in routine practice. IHC is mandatory for HCA subtyping: markers are presented in grey square with their results positive (+) or negative (−) in tumor (T) and nontumoral liver (NT). LFABP: liver fatty acid binding protein; CRP: C-reactive-protein. Final diagnosis of HCA subtypes is: HNF1a inactivated (H-HCA), inflammatory (IHCA), B-catenin activated (B-HCA), B-catenin activated inflammatory (B-IHCA), or unclassified (UHCA). [a]: GS negativity or positivity limited at the periphery and/or around some veins within HCA. [b]: serum amyloid A staining is usually less sensitive and specific than CRP. [c]: aberrant B-catenin nuclear staining. [#]: needs molecular biology confirmation. [d]: can be difficult to differentiate from FNH.

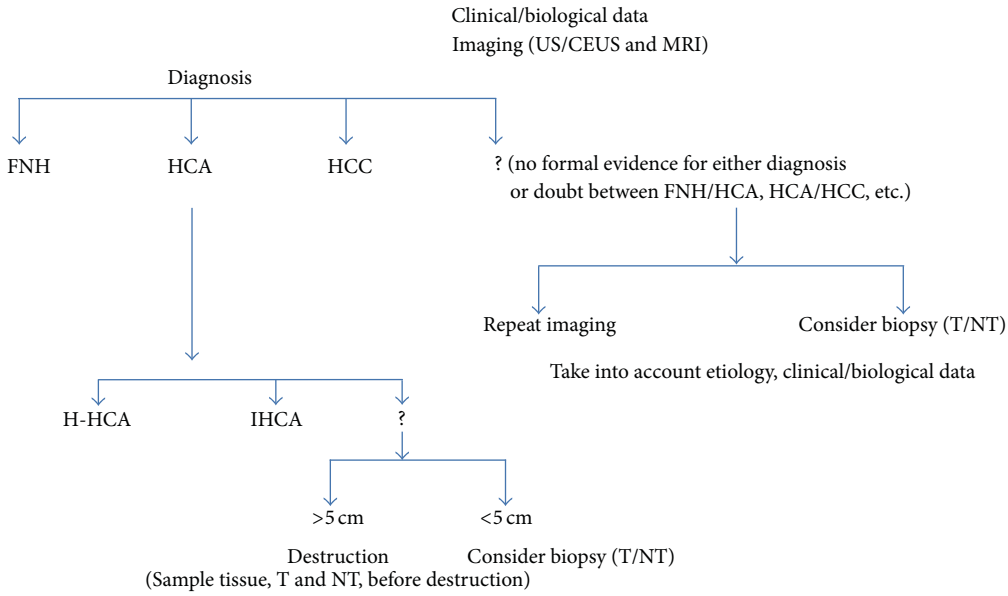
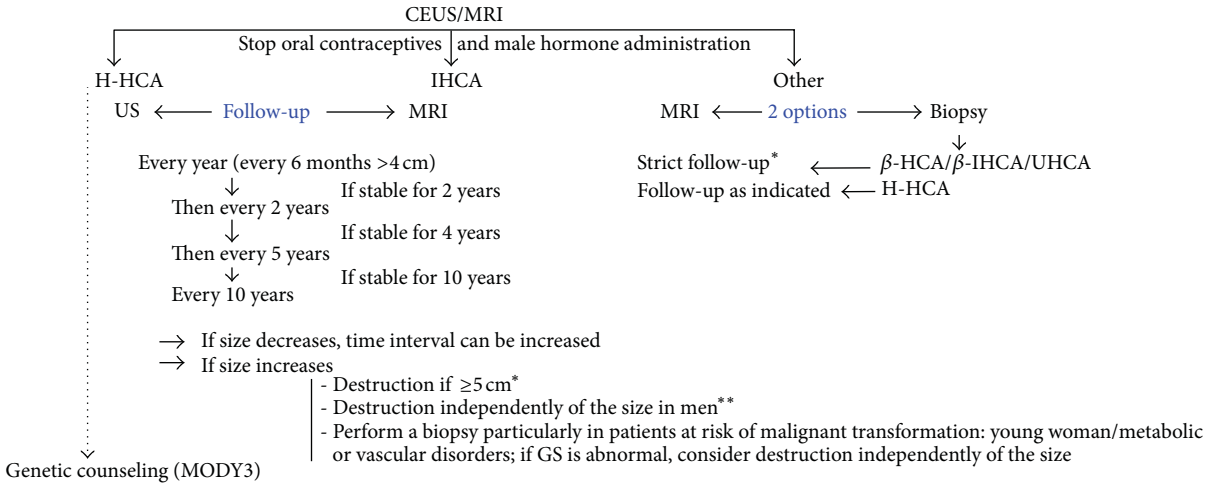


Figure 2: Practical guidelines for the identification of HCA subtypes (outside the emergency context).



In presence of multiple HCA nodules: generally all nodules are of the same subtype (i.e., H-HCA, IHCA). Among IHCA some may be β-catenin mutated, others not. Association of H-HCA and IHCA has been rarely observed in a same liver. In glycogenosis, different types of nodules may exist (β-HCA, IHCA, β-IHCA and UHCA).The destruction (resection, radio frequency) of nodules depends on the size, on the presence of β-catenin mutation and on the disease background. OLT is exceptional and should not be considered just on the number criteria.

*Destruction should be considered for nodules <5 cm (in the 3-4 cm range)

**In men destruction is planned well before the size of the nodule reached 5 cm

Figure 3: Management of HCA subtypes <5 cm.

routine histopathologic and IHC data considering the new molecular data (work in progress).

Because HCA is rare, it is necessary to combine data from different centers. The main task is to better define patients at risk of malignant transformation [58, 59], to understand the susceptibility of some individuals, compared to controls, to develop nodules, either one while others develop myriads, the susceptibility of some nodules to grow while others remain undetectable, to transform while others remain quiescent, and to regress or not. Other main objectives are to study on a

Table 2: IHC in benign liver nodules.

(a) LFABP

	T	NT
H-HCA	− (a)	+ (b)
β-HCA, IHCA, β-IHCA, UHCA	+	+
FNH	+	+
MNR/MNR-FNH-like (cirrhosis)	+	+

(a) Some hepatocytes may be positive at the periphery of the nodule, as well as in between 2 coalescent nodules.
(b) When the expression is weak, reading may be difficult.

(b) CRP

	T	NT
IHCA, β-IHCA	+ (a)	− (b)
H-HCA, β-HCA, UHCA	−	− (b)
FNH	− (b′)	− (b)
MRN/MRN-HNF-like (cirrhosis)	(c)	(d)

(a) Staining can be heterogeneous.
(b) Can be positive (in case of hemorrhage/necrosis, inflammatory syndrome; portal branch embolization, etc.).
(b′) Can be positive if (b) is positive.
(c) Often positive (weak/mild).
(d) Negative to positive (weak to mild), heterogeneous from nodule to nodule.

(c) GS

	T	NT
FNH	+ (a)	(b)
MRN/MRN FNH-like (cirrhosis)	(c)	(d)
β-HCA, β-IHCA	+ (e)	(b)
H-HCA, IHCA, UHCA	−	(b)

(a) Map-like pattern.
(b) Normal positivity around central veins (1–3 rows).
(c) From absence to positivity (limited to veins and/or border of fibrous axis).
(d) Negative, occasional faint staining.
(e) Strong and diffuse or heterogeneous.

large scale HCA in various etiological conditions particularly in vascular diseases and in different liver diseases with different pathological backgrounds particularly NASH and cirrhosis [60].

Deciphering the molecular pathways leading to nodule formation, one can imagine that the medical treatment of HCA is not out of hand.

5. Conclusion

The diagnosis and prognosis of benign hepatocellular nodules have changed in the recent years. This will have an impact on the management of these nodules including surveillance.

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Review Article

MR Imaging of Hepatocellular Adenomas and Differential Diagnosis Dilemma

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Hepatocellular adenomas (HCAs) are currently categorized into distinct genetic and pathologic subtypes as follows: inflammatory hepatocellular adenoma, hepatocyte-nuclear-factor-1-alpha (HNF-1α-mutated) hepatocellular adenoma, and β-catenin-mutated hepatocellular adenomas; the fourth, defined as unclassified subtype, encompasses HCAs without any genetic abnormalities. This classification has accepted management implications due to different risks of haemorrhage and malignant transformation of the four subtypes. Imaging guided biopsy and/or surgical resection very important in obtaining definitive characterization; nevertheless, MRI with intra-extravascular and hepatobiliary (dual phase) agents, is an important tool not only in differential subtypes definition but even in surveillance with early identification of complications and discovery of some signs of HCA malignant degeneration. Inflammation, abnormal rich vascularisation, peliotic areas, and abundant fatty infiltration are pathologic findings differently present in the HCA subtypes and they may be detected by multiparametric MRI approach. Lesion enlargement and heterogeneity of signal intensity and of contrast enhancement are signs to be considered in malignant transformation. The purpose of this paper is to present the state of the art of MRI in the diagnosis of HCA and subtype characterization, with particular regard to morphologic and functional information available with dual phase contrast agents, and to discuss differential diagnosis with the most common benign and malignant lesions mimicking HCAs.

1. Introduction

Hepatocellular adenoma (HCA) is a rare benign tumour (incidence of 1/1,000,000) that is mainly found in women of child-bearing age (second most frequent hepatocellular tumor in young women after focal nodular hyperplasia). There is evidence that HCA is strongly related to current and recent (first generation, high dose) oral contraceptives (OC) use. Recent, low-dose OCs appear less strongly, at all, related to HCA [1]. Sometimes tumour regression has been noted after discontinuation of OC. Non-OC-related causes of HCA include familial insulin-dependent diabetes, Fanconi anaemia, glycogen storage diseases, and hormonal stimulation from other sources, for instance, anabolic steroid use by body builders, gynaecological tumours, or pregnancy [2–6].

Small HCA is generally asymptomatic. Right upper abdominal quadrant fullness or discomfort is present in 40% of cases due to mass effect. Typical clinical manifestation is spontaneous rupture or haemorrhage leading to acute abdominal pain with possible progression to hypotension and even death. HCA rarely undergoes malignant transformation to hepatocellular carcinoma (HCC). Laboratory tests for liver function are usually normal. Because of the different therapeutic options HCA must be distinguished from other hypervascular lesions (HCC, fibrolamellar HCC, focal nodular hyperplasia (FNH), and metastases) that may occur in young adults without cirrhosis.

Steroid related adenoma is typically detected when it reaches about 5 cm in diameter but the size could be 6–30 cm (large and multiple lesions are more prone to spontaneous haemorrhage). In 75% of cases the lesion involves

the subcapsular region of right lobe of liver, in 10% it is intraparenchymal or pedunculated.

HCA is usually solitary (70–80%) and multiple in 20–30%. A so-called adenomatosis is present in subjects observed to have more than 10 lesions: this entity is independent of gender or hormone therapy [7, 8].

Treatment criteria include number and size of lesions, presence of symptoms, and surgical risk. The possible role for elective surgery mainly depends upon the risk of complications, but data concerning its actual incidence are presently lacking. In fact, both selection and spectrum bias negatively affect the retrospective surgical case series that represent most of the available literature on this topic. Moreover, concerning the two other common indications for surgery, represented by the uncertainty of diagnosis and the presence of symptoms related to tumour size, the role of surgery can be challenged. In fact, the continuous improvement in diagnostic techniques, particularly magnetic resonance, has the consequence that one can restrict surgical resection to very few participants for which the procedure has not only a curative target, but also the need for a precise pathologic definition. Also the role of surgery for symptomatic tumors has been claimed only by small uncontrolled case series [9]. However, as a general rule, if HCA is <5 cm in size, discontinuation of OC and radiological follow-up are acceptable; if the lesion is >5 cm or near hepatic surface, due to the recognized risk of rupture and haemorrhage, surgical resection is the treatment of choice. Pregnancy should be avoided due to increased risk of rupture.

According to recent studies HCAs are currently categorized in four distinct genetic and pathologic subtypes as follows: inflammatory hepatocellular adenomas, hepatocyte-nuclear-factor-1-alpha- (HNF-1α-mutated) hepatocellular adenomas, and β-catenin-mutated hepatocellular adenomas. Finally HCAs without any genetic abnormalities are categorized in the unclassified subtype [10–13]. Although this classification is new and not yet widely accepted, it has definitive management implications. Image-guided biopsy or surgical resection with histopathologic and immunohistochemical analysis is necessary for complete characterization of HCAs but MR imaging plays an important role in diagnosis and subtype characterization as well as identification of complications and surveillance [14, 15].

The purpose of this paper is to present MR imaging characteristics of specific subtypes of HCAs by using dual phase contrast agents and to discuss differential diagnosis with the most common benign and malignant lesions mimicking HCAs.

2. Intra- and Extravascular and Hepatobiliary Agents

Gadobenate dimeglumine (Gd-BOPTA, Multihance, Bracco) and gadoxetic acid (Gd-GD-EOB-DTPA, Primovist, Bayer) differ from purely extracellular gadolinium agents as they combine the properties of conventional nonspecific gadolinium agents with those of an agent targeted specifically to hepatocytes: for this reason they are also called *hepatospecific* contrast agents. With these contrast media it is possible to perform dynamic phase imaging as performed with conventional gadolinium agents and delayed-phase imaging as performed with mangafodipir trisodium (Teslascan, GE Healthcare). Using Gd-BOPTA arterial, portal venous, and equilibrium phase images are readily obtainable using identical sequences, the same contrast speed injection to those employed with nonspecific gadolinium agents. In a different way, considering the very fast hepatocyte intake of Gd-EOB-DTPA, the equilibrium phase is spurious because of the overlap between interstitial and hepatocyte times. Indeed, hepatocyte phase after Gd-EOB-DTPA starts after 3 minutes of contrast medium injection. There is an overlap between equilibrium and early hepatocyte phases; therefore, equilibrium appearance after Gd-EOB-DTPA is spurious.

Unlike the conventional agents, approximately 3–5% and 50%, respectively, of the injected doses of Gd-BOPTA and Gd-EOB-DTPA are taken up by functioning hepatocytes and ultimately excreted via the biliary system. The result of the hepatocytic uptake is that the normal liver parenchyma shows strong enhancement on delayed T1w images that is maximal approximately 2-3 hours after Gd-BOPTA injection and 20 minutes after Gd-EOB-DTPA administration. A second feature unique to Gd-BOPTA and Gd-EOB-DTPA is that the contrast-effective moiety of these agents interacts weakly and transiently with serum albumin. This interaction shows the tumbling rate of the Gd-BOPTA and Gd-EOB-DTPA chelates and results in longer rotation correlation times in shell water protons for Gd-BOPTA and Gd-EOB-DTPA compared to generic gadolinium agents that do not interact with serum albumin. This in turn results in a T1 relaxivity in human plasma at 37°C at 1.5 T that is approximately that of conventional gadolinium agents (r_1 7, 3 and 6, 7 for Gd-EOB-DTPA and Gd-BOPTA, resp., in comparison to 4, 2–4, 6 with conventional Gd-chelates) [16]. Not only does this increased relaxivity permit lower overall doses to be used to acquire the same information of dynamic as available with conventional agents at a standard dose, but it also facilitates the improved performance of the Gd-BOPTA and Gd-EOB-DTPA for both intra- and extrahepatic vascular imaging.

A principal advantage of the selective uptake by functioning hepatocytes is that the normal tissue enhances while tumors of nonhepatocytic origin such as metastases and cholangiocarcinoma, as well as nonfunctioning tumor that are unable to uptake Gd-BOPTA and Gd-EOB-DTPA, remain unenhanced, thereby increasing the liver contrast-noise ratio (CNR) and hence the ability to detect lesion.

MR imaging is typically performed, being Gd-BOPTA and Gd-EOB-DTPA T1 relaxation time contrast agents, with 2D or 3D Gradient-Echo sequences while the use of fat saturation has been shown to raise CNR on dynamic and delayed hepatobiliary phase imaging.

Clinical studies and routine clinical practice have shown that the dynamic phase imaging is particularly important for lesion characterization while delayed phase imaging in the hepatobiliary phase increases the sensitivity of MRI for liver detection. However, delayed phase imaging also contributes to improving the characterization of lesions, particularly when results of unenhanced and dynamic sequences are

equivocal or when atypical enhancement patterns are noted on dynamic imaging [17–21].

3. Characterization of HCAs Subtypes with MRI

3.1. Inflammatory HCAs. Inflammatory HCA is the most common subtype and accounts for about 30%–50% of all hepatocellular adenomas. These tumors are mainly seen in women, in association with obesity, hepatic steatosis, diabetes mellitus, glycogenesis (in particular, type I glycogen storage diseases), and alcohol abuse. More than 90% of women have a history of contraceptive use [22]. Patients with inflammatory HCA may present with signs of chronic anemia and/or "systemic inflammatory syndrome," characterized by fever, leukocytosis, and elevated serum levels of C-reactive protein [23]. Inflammatory HCAs are associated with a definitive increased risk of bleeding (>30%) and a risk of malignant transformation (5–10%) [10, 12]. They comprise a prototype example of tumours induced by hepato-biliary inflammation: more than 40% of genes overexpressed in inflammatory HCAs are associated with inflammation and immune response. Around 10% of inflammatory HCAs may also show mutation involving β catenin gene.

Histologically, inflammatory HCAs are characterized by significant sinusoidal dilatation, polymorphous inflammatory infiltrates, areas of peliosis, and thickened tortuous arteries. Prominent ductal reaction represents the distinct histological feature. Steatosis within nodule is variable and less extensive compared with HNF-1α-mutated HCAs [15, 24].

On plain MR imaging inflammatory HCA is often hyperintense on T2w images and hypointense on T1w sequence, frequently with heterogeneous signal intensity. Hyper- and hypointensity on T2w and T1w images, respectively, correspond mainly to areas of sinusoidal dilatation and inflammatory infiltrates. Focal areas of fat may be seen as hypointense areas on T1 out-phased images due to signal drop. Inflammatory HCA may appear as a hypervascular mass with persistent enhancement during dynamic evaluation and may show a variable uptake in the hepatobiliary phase specially at the periphery (Figure 1). Sometimes because of sinusoidal dilatation, inflammatory component and ductal reaction, in the hepato-biliary phase image areas of hypointensity in adenomas, mainly in the periphery, may be seen [25, 26]. Marked T2 hyperintensity associated with delayed persistent enhancement has a sensitivity of as much as 85% and a specificity of 87% for the diagnosis of inflammatory HCA. Peripheral hyper-intensity (*atoll sign*) reflects the abnormal ductal reaction with alterated biliary excretion (Figure 2) [13, 14]. In a small percent of cases, inflammatory HCA may appear isointense on T2 and T1w images with discrete enhancement in arterial phase and quite rapid wash out (Figure 3).

3.2. HNF-1α-Mutated HCAs. HNF-1α-mutated HCA is the second most common type; it constitutes about 30–35% of all HCAs and arises because of biallelic inactivation of transcription factor 1 gene located in chromosome twelve. This kind of adenoma is nearly exclusively seen in women, except for rare HCA with germline HFN-1α mutations which can be also observed in men. Hepatocyte nuclear 1α mutation may be somatic or less frequently germline in origin. The final outcome of this mutation is the production of nonfunctioning HNF-1α protein which promotes lipogenesis by suppression of gluconeogenesis, activation of glycolysis, and promotion of fatty acid biosynthesis. The reduction of fatty acid binding protein 1 leads to faulty transport of fatty acids and to intracellular deposition of fat. Indeed, HNF-1α-mutated HCA is characterized by diffuse intralesional steatosis. HNF-1α mutation may be the primary inciting event that results in the accumulation of estrogen metabolites that unconditionally stimulate hepatocyte proliferation [27, 28].

On MR examination, HNF-1α-mutated HCA often shows heterogeneous hypointensity areas on T1 out-phased sequences with significant signal drops on out-phased in comparison with in-phased sequences, corresponding to fatty deposition. Hyperintensity on T1 in-phased and out-phased images signal drop may correspond to glycogen component or less commonly haemorrhage. On T2w images the lesion tends to appear as iso- or hypointense nodule without significant restriction on DWI. This is true in uncomplicated adenomas; conversely, complicated adenomas or adenomas containing different tissues may show restriction. ADC maps may appear aspecific, with positive or negative values between 0.9 and 1.3 [29, 30].

On dynamic evaluation after Gd-BOPTA and Gd-EOB-DTPA, HNF-1α-mutated HCA appears hypervascular with variable degrees, but usually less evident than inflammatory adenoma. On portal venous and equilibrium phases the lesion tends to be hypointense; on hepatobiliary phase the mass appears hypointense in almost all cases with homogeneous appearance (Figure 4).

Significant signal drop in T1 out-phased imaging for predictive HNF-1α-mutated HCA is reported to be 85%, 100%, 100%, 94% of sensitivity, specificity, predictive positive, and negative predictive value, respectively [14].

3.3. β-Catenin-Mutated HCAs. β-Catenin-mutated HCAs constitute about 10–15% of all HCAs; they originate from sustained activation of β-catenin because of mutations involving the CTNNB1 gene (catenin β1). These tumors primarily involve patients with glycogen storage disease and on androgen treatment and have a greater propensity to undergo malignant transformation to HCCs. β-Catenin plays a major role in hepatocyte development, differentiation, proliferation, and regeneration. Activation of β-catenin in normal hepatocyte is usually transient and is regulated by its rapid degradation. Excessive nuclear accumulation and sustained activation of β-catenin may result from mutation in β-catenin itself or from mutation involving cytoplasmic degradation complex. Excessive β-catenin activity results in autonomous growth of hepatocyte and accelerates HCA formation [22, 27, 31].

Although β-catenin mutation is implicated in malignant transformation of HCA, only 20–30% of malignant HCAs

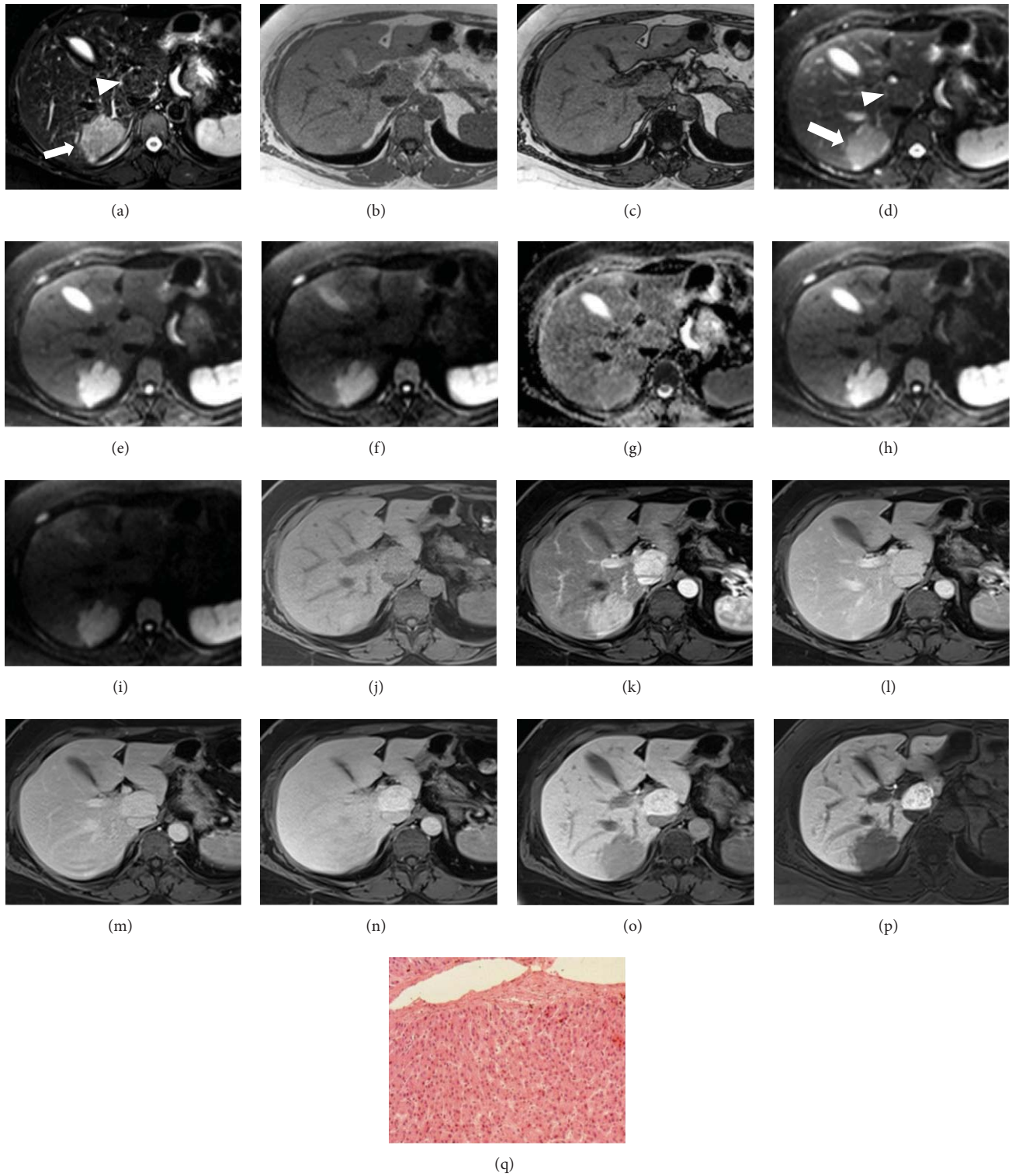

Figure 1: Inflammatory adenoma and focal nodular hyperplasia. (a) Axial T2w fat-suppressed image. In Segment (S) VII homogeneous well-delineated hyperintense adenoma (arrow), proven by biopsy. In SI, note a second isointense lesion, focal nodular hyperplasia (arrowhead). (b-c) T1w in- and out-phased images. Both lesions are mainly isointense. (d–i) DWI sequences. Adenoma (arrow in d) is constantly hyperintense. Conversely FNH (arrowhead in d) is isointense. ADC map (i) both lesions are is slightly hyperintense. (j–n) In dynamic evaluation after Gd-EOB-DTPA administration adenoma shows intense enhancement in arterial phase (k) with washout in portal (l) and equilibrium (m) phases; conversely, FNH shows progressive increase of the signal. (o-p) In hepatobiliary phases after $10'$ (o) and $20'$ (p), adenoma is hypointense relative to the adjacent liver parenchyma and FHN is hyperintense. (o) Histology shows hepatocytes arranged in plates that are two to three cells thick separated by sinusoids.

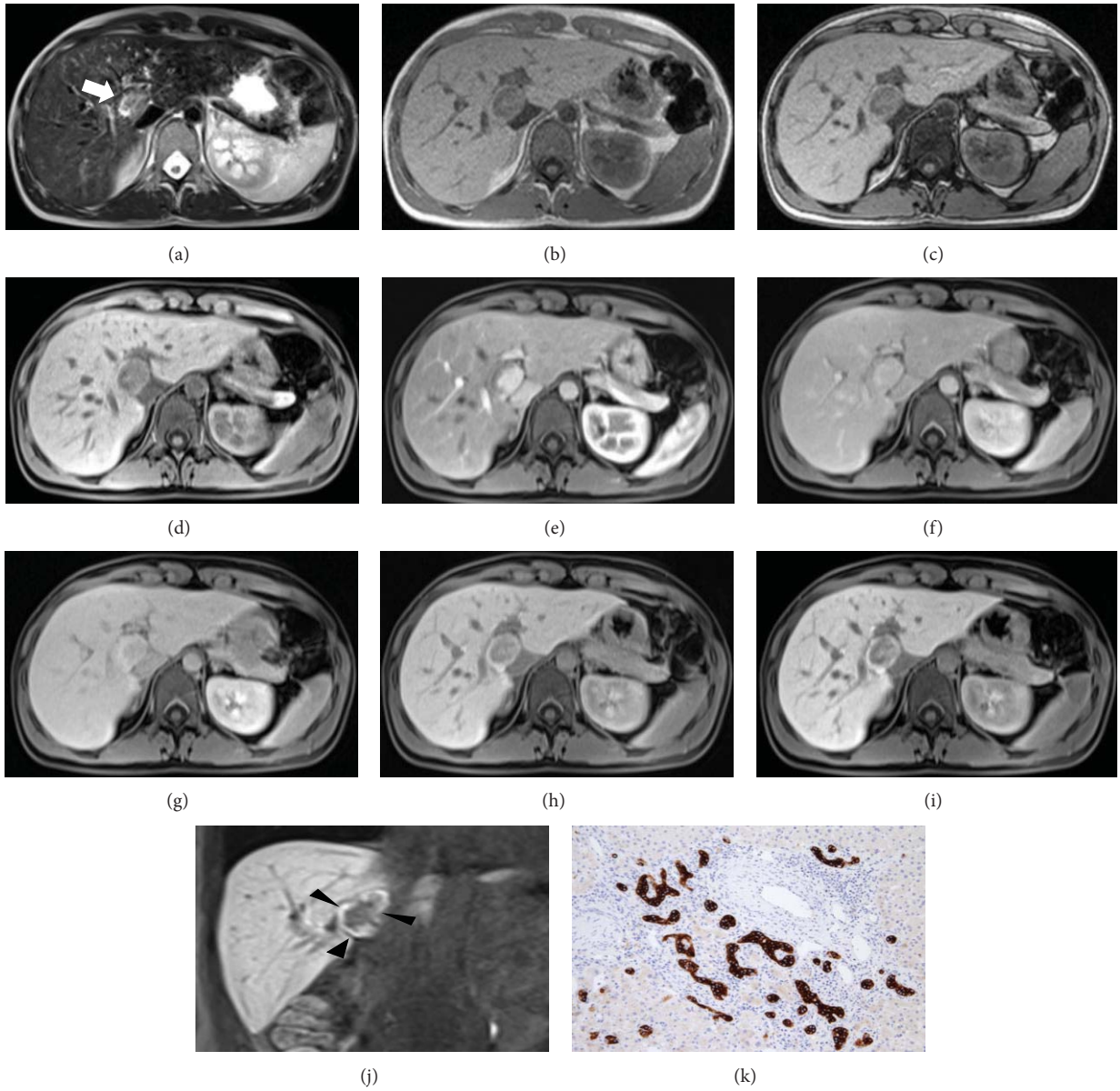

Figure 2: Inflammatory adenoma with "atoll sign." (a) In S IX well-delineated slightly hyperintense lesion with hyperintense peripheral rim (arrow) in axial T2w image. (b-c) T1w in- and out-phased images. The mass is hypointense relative to the hepatic parenchyma with isointense rim. (d–h) In dynamic evaluation after Gd-EOB-DTPA administration the lesion shows marked central enhancement in arterial phase (e) with slight wash out in portal (f) and more evident equilibrium (g) phases. After 5′ (h) the nodule is mainly hypointense. Conversely, the peripheral component of the nodule demonstrates progressive enhancement over time. (i-j) In hepatobiliary phases after 20′, axial (i) and coronal (j), the lesion is heterogeneously hypointense in the central portion and hyperintense at the periphery (arrowheads). Final diagnosis was obtained by biopsy. Histology demonstrates ductal reaction at the periphery of the lesion (k).

show β-catenin mutation. Glycogen storage disease is an additional independent risk for malignant HCA transformation. Up to 75% of patients, glycogen storage disease may develop HCA [32].

On MR imaging β-catenin-mutated HCA appears as homogeneous or heterogeneous hypervascular mass with persistent or nonpersistent enhancement during the delayed-phase images. Signal intensity on T2 and on T1 precontrast sequences is variable but mainly heterogeneously hyper-and hypointense, respectively. Malignant transformation simulates HCC on imaging and does not show peculiar findings (Figure 5) [14].

3.4. Unclassified HCAs. Approximately 10% of all HCAs are without specific genetic and/or pathologic abnormalities. Frequently, the presence of haemorrhage may be one of the reasons that justify the unclassified categorization of the

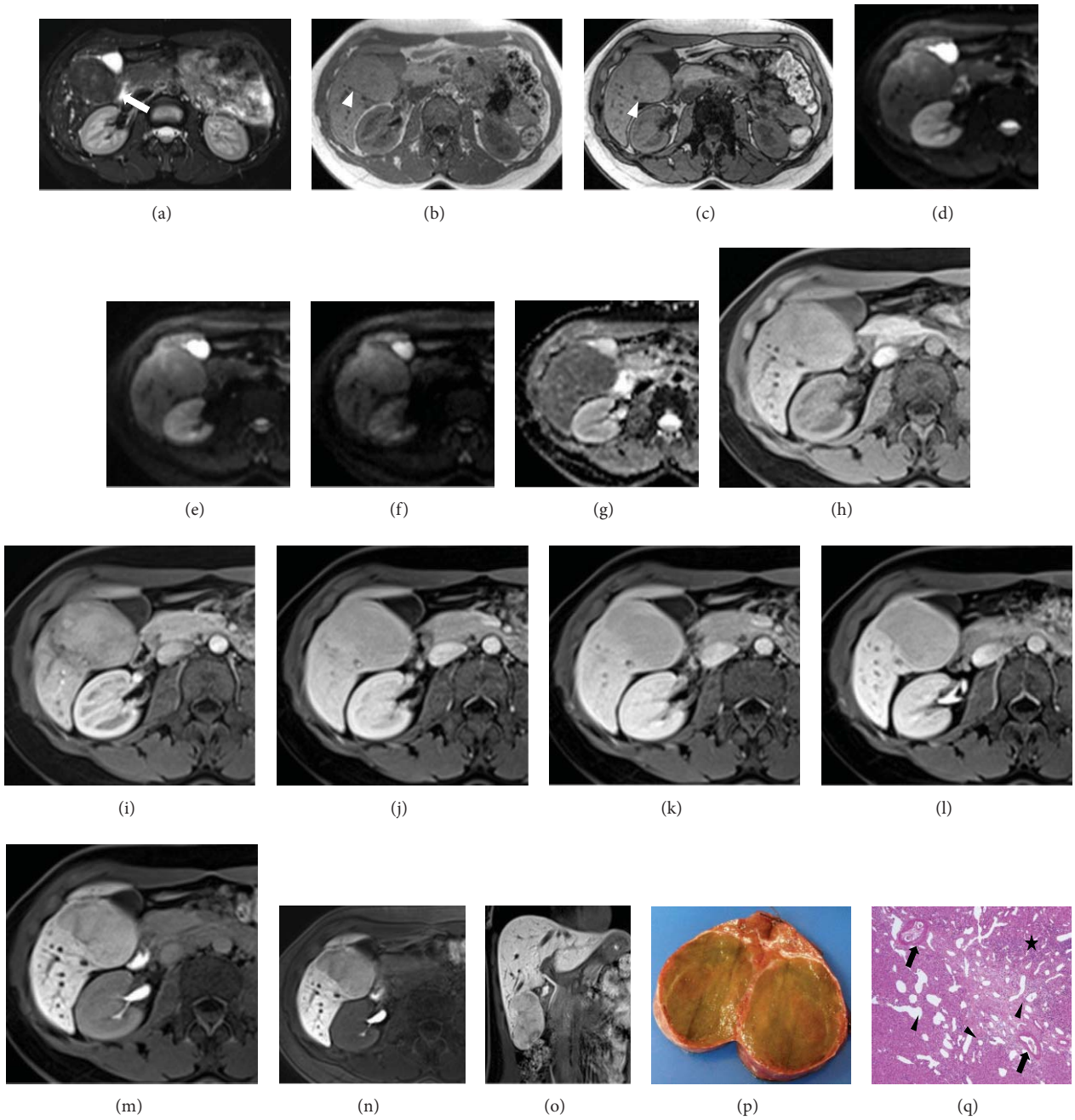

FIGURE 3: Atypical inflammatory adenoma. (a) In S VI homogeneous well-delineated isointense lesion in axial T2w fat-suppressed image (arrow). (b-c) T1w in- and out-phased images. The mass is isointense relative to the hepatic parenchyma. Note large vessels at the periphery of the lesion (arrowheads). (d–g) DWI sequences. The lesion does not show any increase of signal intensity from 50 (d) to 400 (e) and to 800 (f) *b* values. IN ADC map (g) the nodule shows isointense signal. (h–l) In dynamic evaluation after Gd-EOB-DTPA administration the mass shows discrete enhancement in arterial phase (i) with slightly wash out in portal (j) and equilibrium (k) phases. After 5′ (l) the nodule is more hypointense. (m-n) In hepatobiliary phases after 10′ (m) and 20′ (n), the lesion is definitively heterogeneously hypointense relative to the adjacent liver parenchyma with exophytic growth. (o) Cut section shows large capsulated homogeneous mass. (p) Microscopically, significant sinusoidal dilatation (arrowheads), polymorphous inflammatory infiltrates (star), areas of peliosis, and thickened tortuous arteries (arrow) are demonstrated.

lesion (Figure 6). No specific MR imaging patterns have yet been proposed to identity unclassified HCAs also because imaging experience is very limited [10, 22].

No immunohistochemical analyses were performed to compare with MRI features, allowing to assert the diagnosis of HCA subtype.

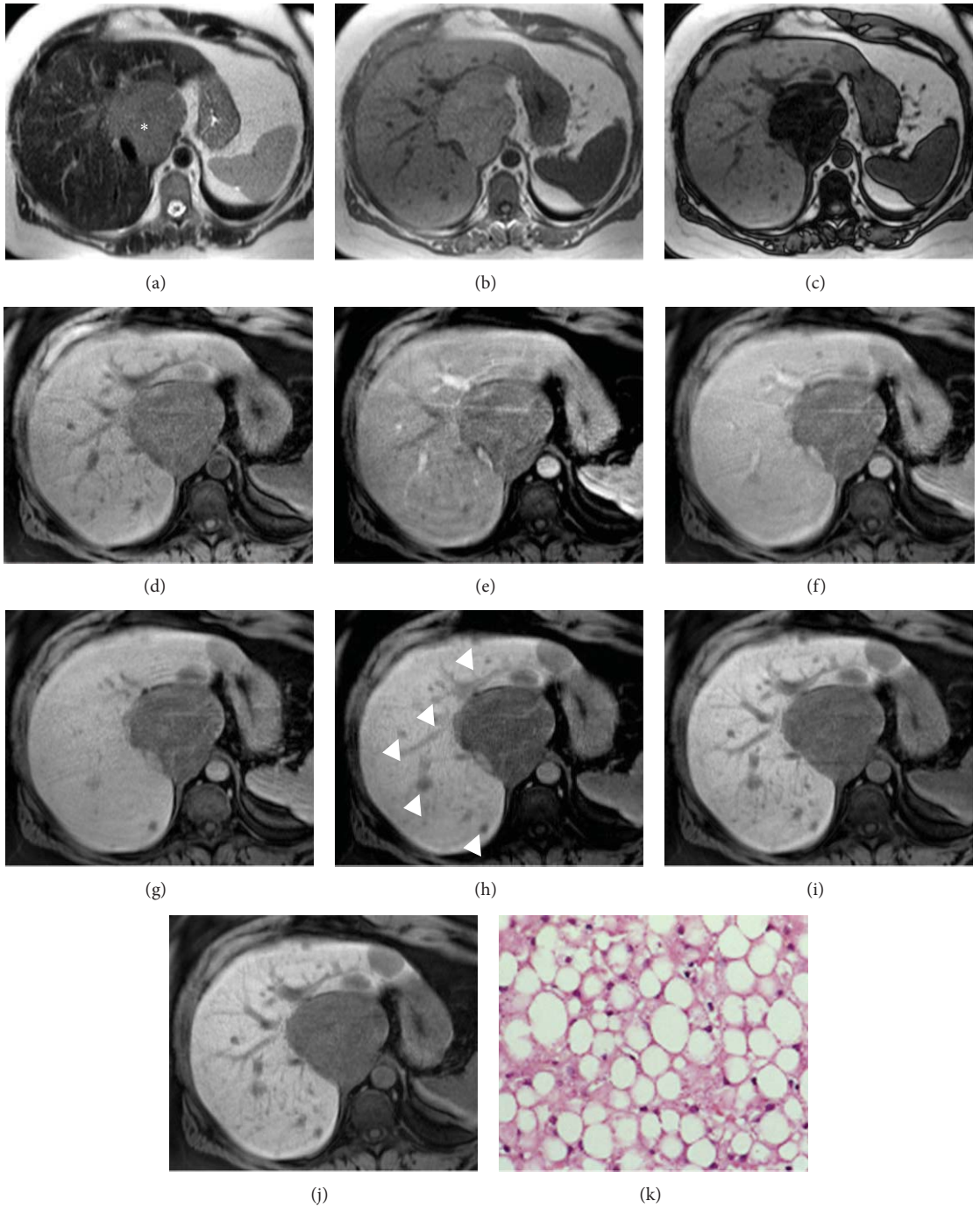

Figure 4: HNF-1α-mutated adenomas. (a) Axial T2w images without fat suppression. In S I and S II multiple slightly homogeneous hyperintense lesions (asterisk indicates the largest nodule biopsied). (b-c) T1w in- and out-phased images. Due to significant intralesional presence of fat tissue, the nodules show signal drop with variable degree in c. (d–h) In dynamic evaluation after Gd-EOB-DTPA administration the nodules show poor enhancement. In (h), after 5′ the nodules are more hypointense; other lesions may be detected in both hepatic lobes (arrowheads). (i-j) In hepatobiliary phases after 10′ (i) and 20′ (j), all adenomas are hypointense relative to adjacent liver parenchyma. (k) Histology confirms presence of fat-rich hepatocytes.

4. Differential Diagnosis Dilemma

Some benign and malignant lesions may simulate HCA. The differential diagnosis depends on clinical and MRI findings.

4.1. Focal Nodular Hyperplasia. Focal nodular hyperplasia is the second most common benign hepatic tumor (8% of primary hepatic tumors at autopsy). FNH represents an hyperplastic response to a localized vascular abnormality;

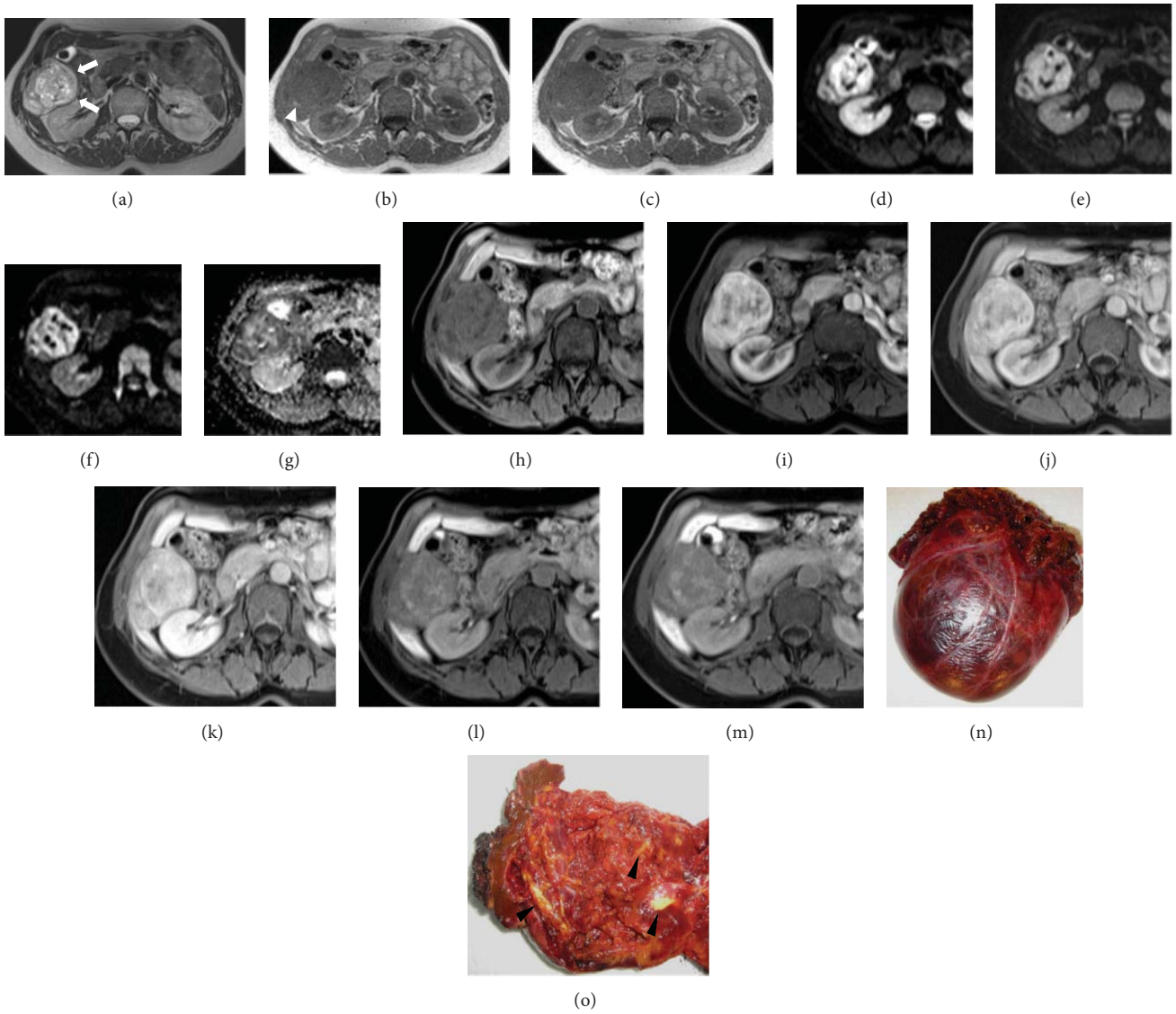

Figure 5: β-catenin-mutated adenoma. (a) In S VI heterogeneous well delineated hyperintense lesion (arrows). (b-c) T1w in- and out-phased images. The mass is slightly hypointense relative to hepatic parenchyma with focal signal drop due to intralesional fatty infiltration (arrowhead). (d–g) DWI sequences. The lesion increases signal intensity from 50 (d) to 400 (e) and to 800 (f) *b* values. In ADC map (g) the nodule shows heterogeneous signal. (h–l) In dynamic evaluation after Gd-EOB-DTPA administration the mass shows intense heterogeneous enhancement in arterial phase (i) without evident wash out in portal (j) and (k) equilibrium phases. (l-m) In hepatobiliary phases after 10′ (l) and 20′ (m), the lesions are heterogeneous hypointense relative to the adjacent liver parenchyma. (n-o) Gross specimen confirms capsulated heterogeneous mass with fatty component (arrowheads).

consequently it is not a true benign tumor but a benign congenital hamartomatous malformation.

FNH is predominantly found in the same group of patients as well as HCA: female patients (M : F = 1 : 8), usually in 3rd-4th decades of life, with history of OC consumption. Multiple FNHs are found in 10–20% of cases and association with hemangioma occurs in 5–10% of cases.

Patients are generally asymptomatic (50–90% incidental finding). Vague abdominal pain (10–15%) due to mass effect may be present. Laboratory tests for liver function are generally normal. Pathologically FNH is usually a solitary (80%), subcapsular, and nodular homogeneous mass [33].

FNHs could be subdivided into FNH with sinusoidal dilatation (most of the so-called telangiectatic FNH are now recognized as inflammatory HCA) and FNH with cytological atypia. On cut section in the majority of large FNHs, pathological features are fibrous septa and cellular areas of hepatic proliferation. Hepatocytes rarely may contain fats, triglycerides, and glycogens. Lesions more than 5 cm frequently show a central fibrous scar which consists of fibrous connective tissue, cholangiocellular proliferation, and malformed vessels (arteries, capillaries, and veins). The capsule is seldom present and margins are usually sharp. Unlike HCA, haemorrhage and necrosis are exceptional within the

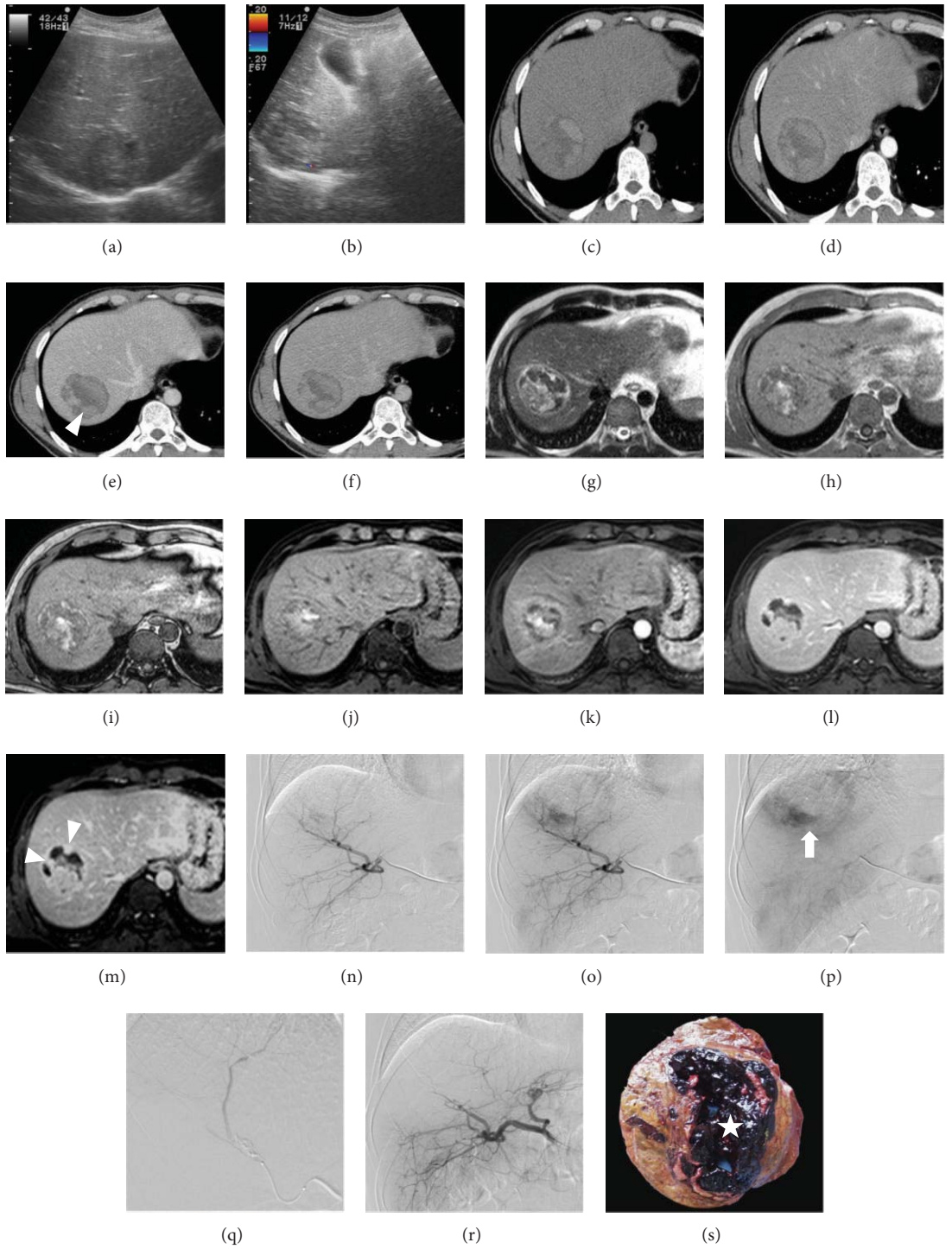

Figure 6: Bleeding adenoma in young man with sudden and acute upper abdominal pain. (a-b) Ultrasound, B-mode (a) and color-Doppler (b) imaging show heterogeneous lesion without significant vascularization. (c–f) CT confirms heterogeneous hyperdense bleeding mass with persistent vascularized tissue in the lower portion of the lesion (arrowheads). (g) Axial T2w image shows mixed heterogenous bleeding capsulated mass (arrows) in S VIII. (h-i) T1w in- and out-phased images confirm intralesional hemorrhage. (j–m) MR dynamic study shows vascularized tissue in the lower portion of the lesion (arrowheads). (n–p) DSA before embolization. Note hypervascular tissue in the lower part of the lesion (arrow). (q-r) DSA after embolization, complete devascularization of the nodule. (s) Cut section of the resected lesion shows large hemorrhagic component (star).

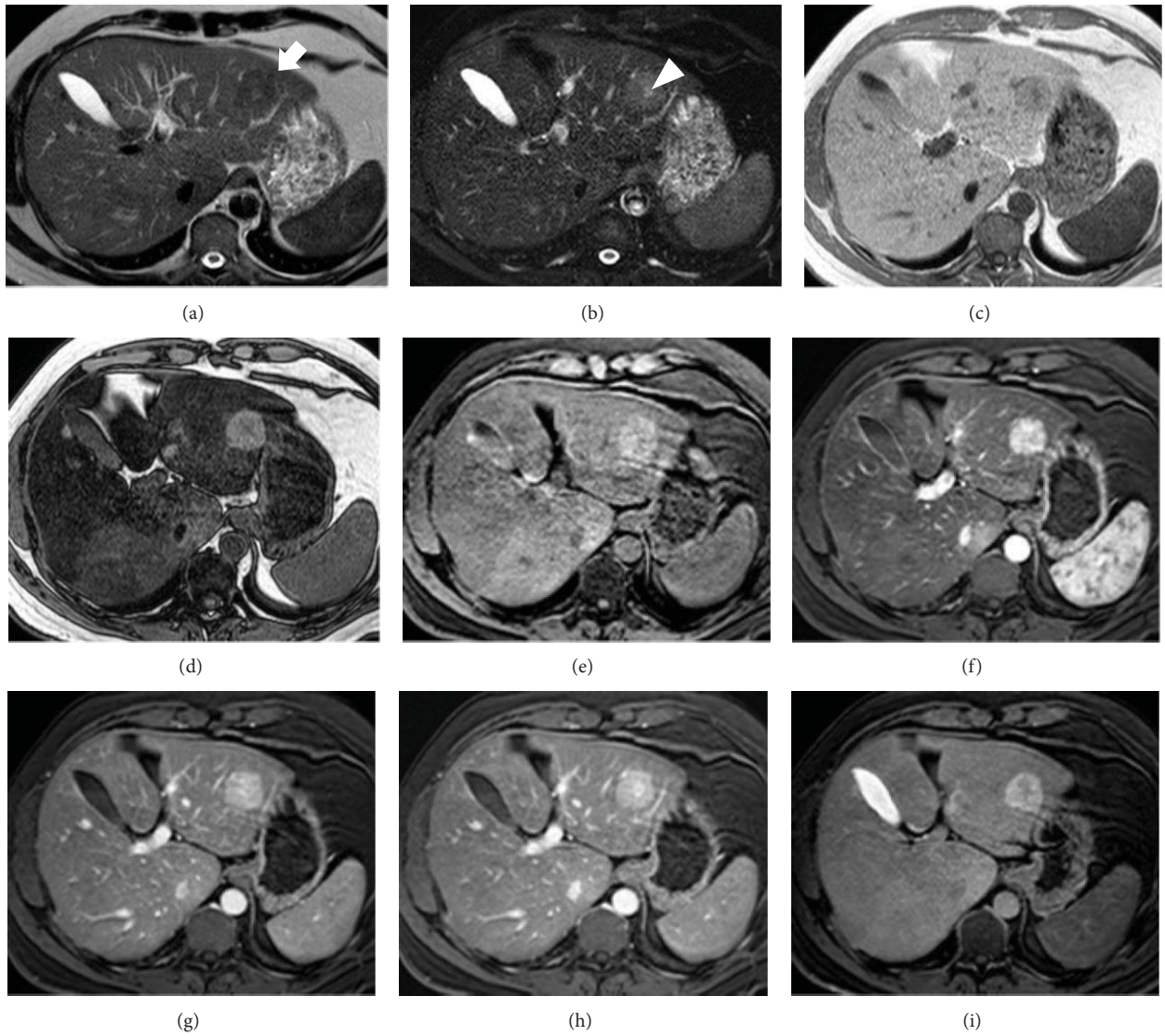

Figure 7: Focal nodular hyperplasia in fatty liver. (a-b) Axial T2w images without (a) and with (b) fat suppression. In S II slightly hypointense (a) and hyperintense (b) lesions (arrow) with hyperintense central scar (arrowhead). (c-d) Due to significant steatosis of hepatic parenchyma the lesion is hypo- and hyperintense in T1w in- and out-phased images. (e–h) In dynamic evaluation after Gd-BOPTA administration the nodule appears significantly hypervascular in arterial phase (f) and tends to be hyperintense in portal and equilibrium phases. Typically central scar becomes hyperintense in equilibrium phase. (i) Hepatobiliary phase image after 1 hour. The lesion is hyperintense except central scar which is hypointense with stellate aspect.

lesion. No malignant degeneration of FNH has been observed [34].

Surgical resection is suggested only in large symptomatic lesions.

On MR imaging, classic FNH appears as homogeneously isointense or slightly hyperintense on T2w images and isointense or slightly hypointense on T1w images before contrast medium administration. Typical behaviours during dynamic study are marked and homogeneous enhancement during the arterial phase, rapid wash out during the portal phase, and isointensity (with the exception of the scar) during the equilibrium phase. In hepatobiliary phase FNH is isointense or slightly hyperintense on T1w images. In contrast, on delayed liver specific phase images after Gd-BOPTA and Gd-EOB-DTPA administration, the common appearance of HCAs is hypointensity of the solid, nonhemorrhagic components of the lesions, with the exception of the inflammatory subtype. This one is the main feature that differentiates FNHs from HCAs lesions. When present, in FNH a typical scar appears as hyperintense or hypointense stellate area, respectively, on T2 and T1-weighted images; it is hypointense during the arterial and portal-venous phases and slightly hyperintense during the equilibrium phase (Figure 7) [35–37].

4.2. Large Regenerative Hyperplasia. Large regenerative hyperplasia (LRH) is another generally asymptomatic

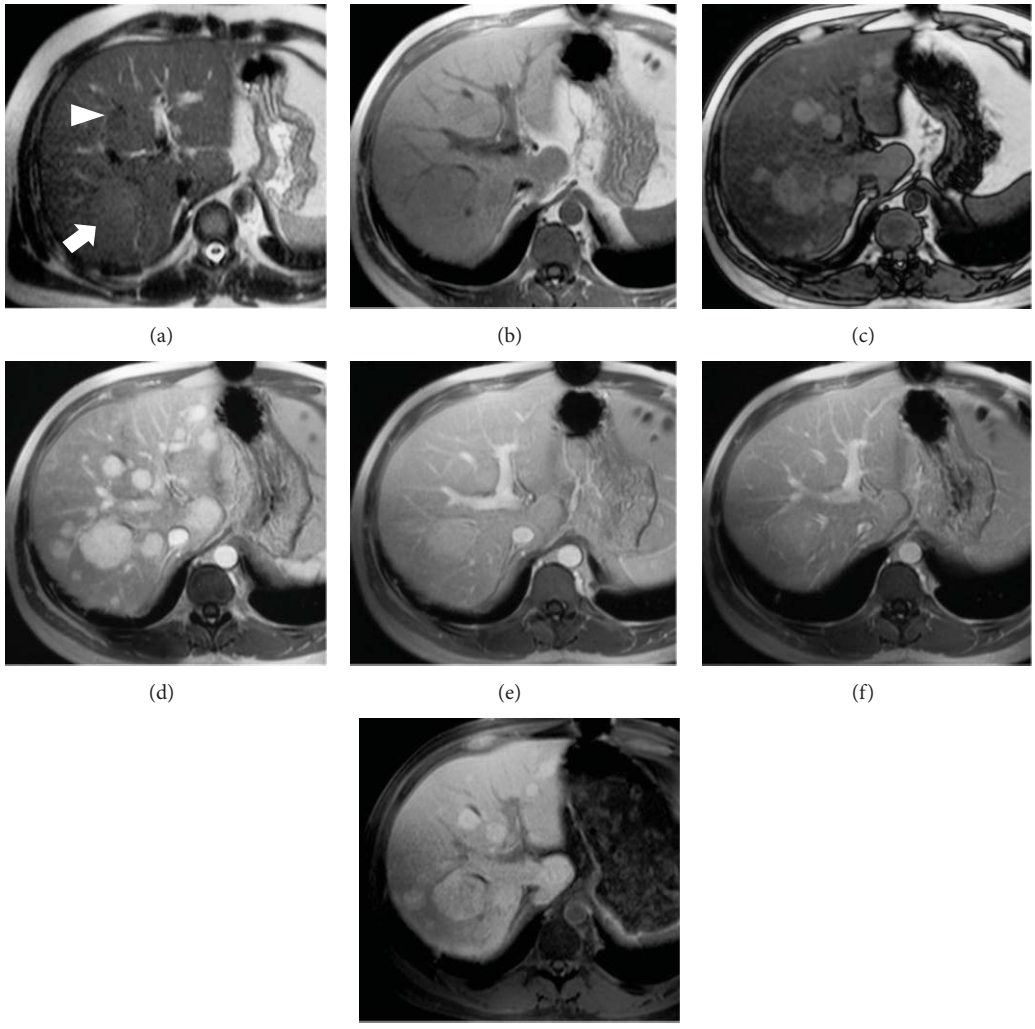

Figure 8: Large regenerative hyperplasia. (a) Axial T2w image. In S VII slightly hyperintense nodule (arrow); in SIV moderate hypointense lesion (arrowhead). (b-c) T1w in- and out-phased images. Due to significant steatosis of hepatic parenchyma after chemotherapy, many other hypointense lesions appear in out-phased sequence. (d–f) In dynamic evaluation after Gd-BOPTA administration the nodules appear significantly hypervascular in arterial phase (d) and tend to be isointense in portal and equilibrium phases. (g) Hepatobiliary phase image after 1 hour. Nodules are hyperintense.

rare condition (0.5–2.5% on autopsy series) characterized by diffuse micronodular transformation of the hepatic parenchyma, without fibrous septa between nodules. Disorders in the hepatic microcirculation (chronic ischemia) with hyperplastic parenchyma response seem to be the primary cause of LRH. Myeloproliferative and lymphoproliferative disorders, chronic vascular and rheumatologic syndromes, and some drugs used are related to LRH. Adults (mean age 50), without gender predilection known, are more affected by LRH, rarely reported in childhood (e.g., in congenital absence of portal vein). Acute abdominal pain occurs in case of rupture of a large subcapsular nodule with hemoperitoneum. If central scar is present nodules can be indistinguishable from FNH. Portal vein obstruction and secondary hepatic arterial dilatation can be observed. In case of splenomegaly and increased flow in portal vein, several nodules can be observed with patent portal branches. When thrombosis of a large portal branch occurs, remnant portal flow is redirected in the perihilum parenchyma with large regenerative nodules (of several cm) near the large portal tracts and small nodules with atrophy in the peripheral parenchyma. No malignant degeneration of LRH has been observed [38, 39].

Prognosis depends on underlying disease. Treatment is indicated in progressive hepatic failure related to underlying disease.

On unenhanced T1w MR images LRH is generally almost isointense or slightly hyperintense compared to the surrounding liver parenchyma while on unenhanced T2w images the nodule appears iso- or slightly hypointense. A pheripheral hypointense rim is often visible in large lesions on T1- and T2-weighted images. On dynamic MR imaging,

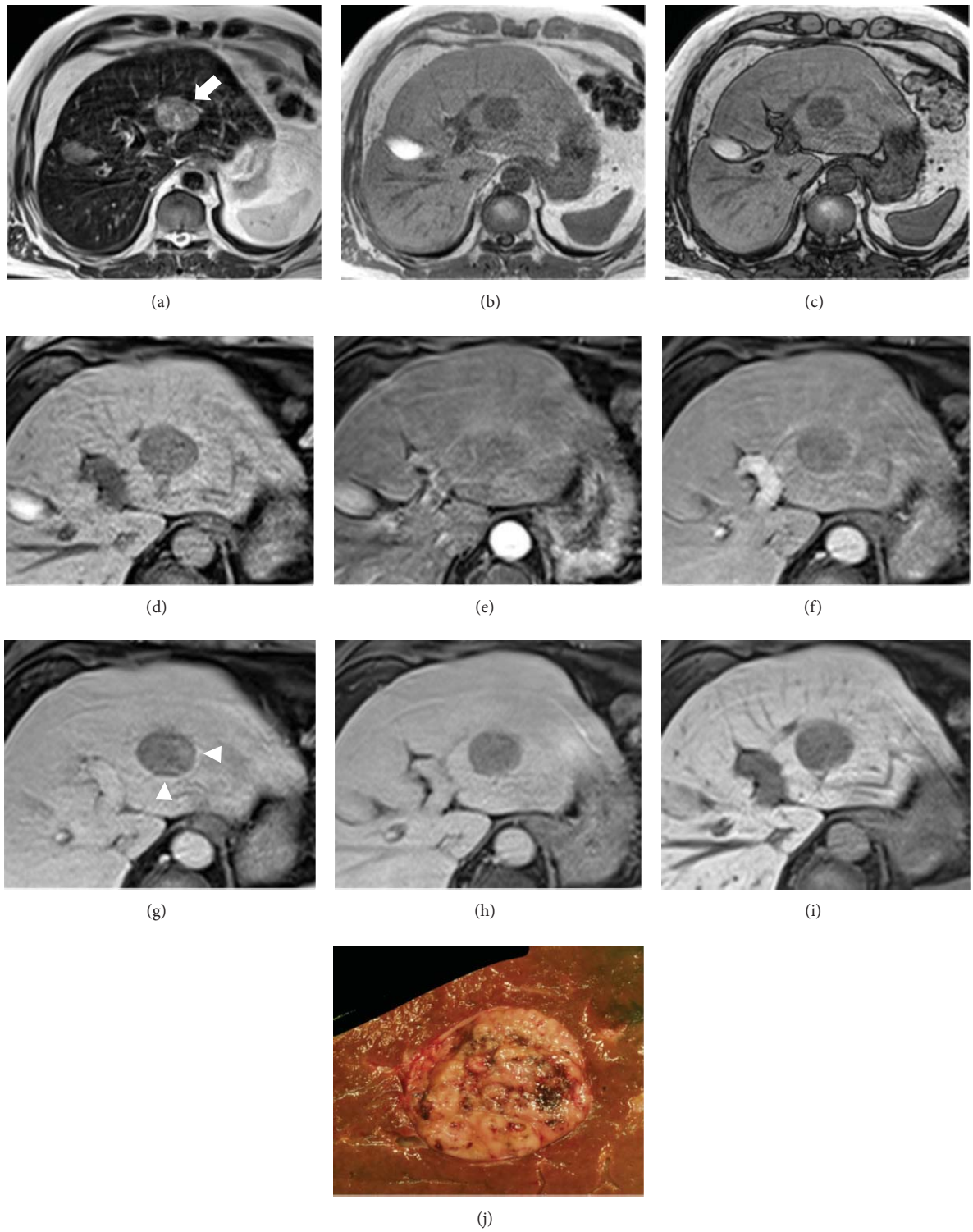

Figure 9: HCC in noncirrhotic liver. (a) Axial T2w image. In S II-S III slightly hyperintense well delineated lesion (arrow). (b-c) In T1w in- and out-phased images of the lesion appears hypointense. (d–h) In dynamic evaluation after Gd-EOB-DTPA administration the nodule appears slightly hypervascular in arterial phase (e); it shows rapid and progressive wash out in portal and equilibrium phases. Note the presence of a pseudocapsule (arrowhead) in equilibrium phase. (i) Hepatobiliary phase image after 20′. The lesion appears hypointense. (j) Pathologic specimen confirms the presence of HCC nodule in normal liver.

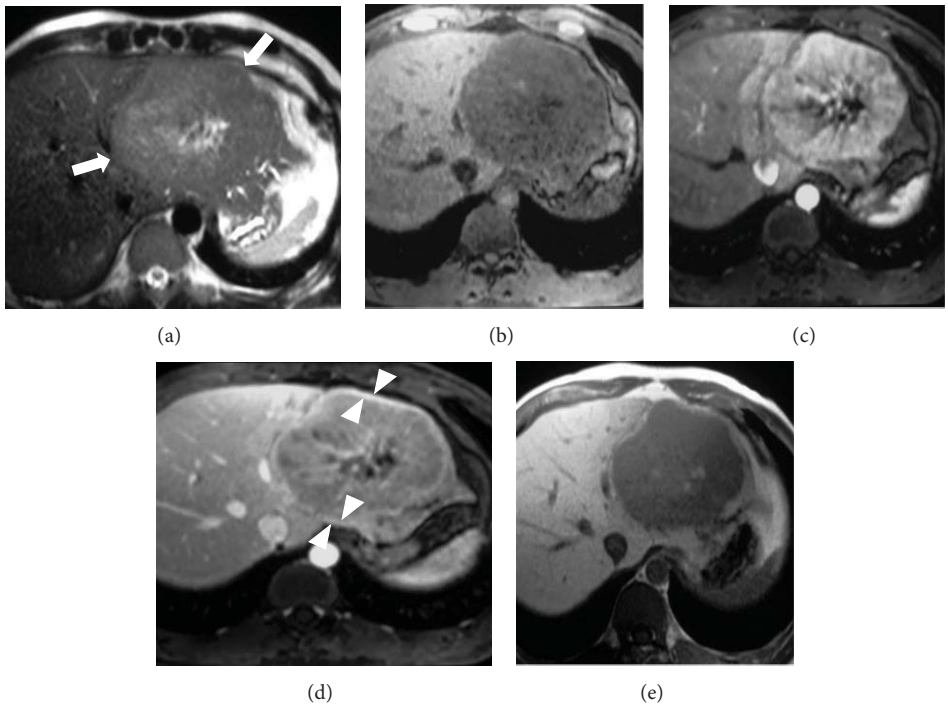

Figure 10: Fibrolamellar HCC. (a) Axial T2w image. In left lobe slightly well delineated mass (arrows) with heterogeneous central area. (b–d) In dynamic evaluation after Gd-BOPTA the lesion appears markedly heterogeneous hypervascular in arterial phase (c); it shows rapid and progressive wash out in portal phase. The central area is hypointense. Note complete thick pseudocapsule at the periphery (arrowhead). (e) In hepatobiliary phase image the lesion is hypointense; the central fibrotic component retains contrast agent.

LRH is usually hyperintense in the arterial phase and iso- or slightly hyperintense in the portal-venous and equilibrium phases. In the delayed, liver specific phase after Gd-BOPTA or GD-EOB-DTPA the lesion appears isointense or hyperintense because it consists of benign hepatocytes with abnormal biliary system drainage (Figure 8) [40, 41].

The main diagnostic dilemma is between LRH and inflammatory HCA; the key elements to achieve the differential diagnosis are the different signal on T2 images (isointense or slightly hypointense in LRH, hyperintense in HCA); the dissimilar contrast behaviour in dynamic study.

4.3. Hepatocellular Carcinoma in Noncirrhotic Patients and Fibrolamellar Hepatocellular Carcinoma. HCC in noncirrhotic patients and fibrolamellar hepatocellular carcinoma (FL-HCC) are rare malignant primary liver tumors which arise in young healthy patients of both sexes (M : F = 1 : 1).

Hepatomegaly, malaise, pain in right upper quadrant, fever, and/or weight loss may be present. The lesion becomes symptomatic only when the mass reaches very big size and compresses adjacent structures or invades vascular or biliary vessels (rarely jaundice can reveal the presence of tumour).

Both tumors can present with metastatic disease (lymph nodes and lungs are the most frequent sites). Alpha-fetoprotein is usually elevated in conventional HCC but often normal in FL-HCC.

Generally there is a large (average mean size >10 cm), single (80–90%), well-demarcated, lobulated, noncapsulated mass (incomplete capsule in 1/3 of cases); in 20% of cases it can be pedunculated.

In FL-HCC coarse calcifications had been depicted in more than 50% of lesions and, at cut section, lobular arrangement fibrous septa with radial disposition and central scar are seen in 60–70% of cases; abdominal lymphadenopathy had been detected in >60% of patients.

Central scar, fibrosis, and calcification are rare in conventional HCC whereas necrosis hemorrhage and intratumoral fat are much more common than in FL-HCC.

The surgical resectability is high (50%); multiple or too large tumors can be treated with liver transplantation. FL-HCC is frequently recurrent, but 5-year survival is about 50–60%.

On MR imaging, HCC in non-cirrhotic patients depends largely on tumor size that is generally large because the lesion is not detected in early stage. Generally, most HCCs, because of their hypervascular nature, are hyperintense compared to the liver in the arterial phase and hypointense in the portal-venous and equilibrium phases. Irregular mosaic-like or pheripheral enhancement is usually seen in large neoplasms, depending on the internal architecture. In the delayed liver-specific phases after Gd-BOPTA or Gd-EOB-DTPA well-differentiated and moderately differentiated HCCs may show in a small percentage of cases superior signal enhancement ratios to poorly differentiated HCCs (Figure 9).

Differential diagnosis between HCC and HCA, subtypes inflammatory and β-catenin, is very difficult: both adenomas may be heterogeneously hyperintense on T2-weighted images

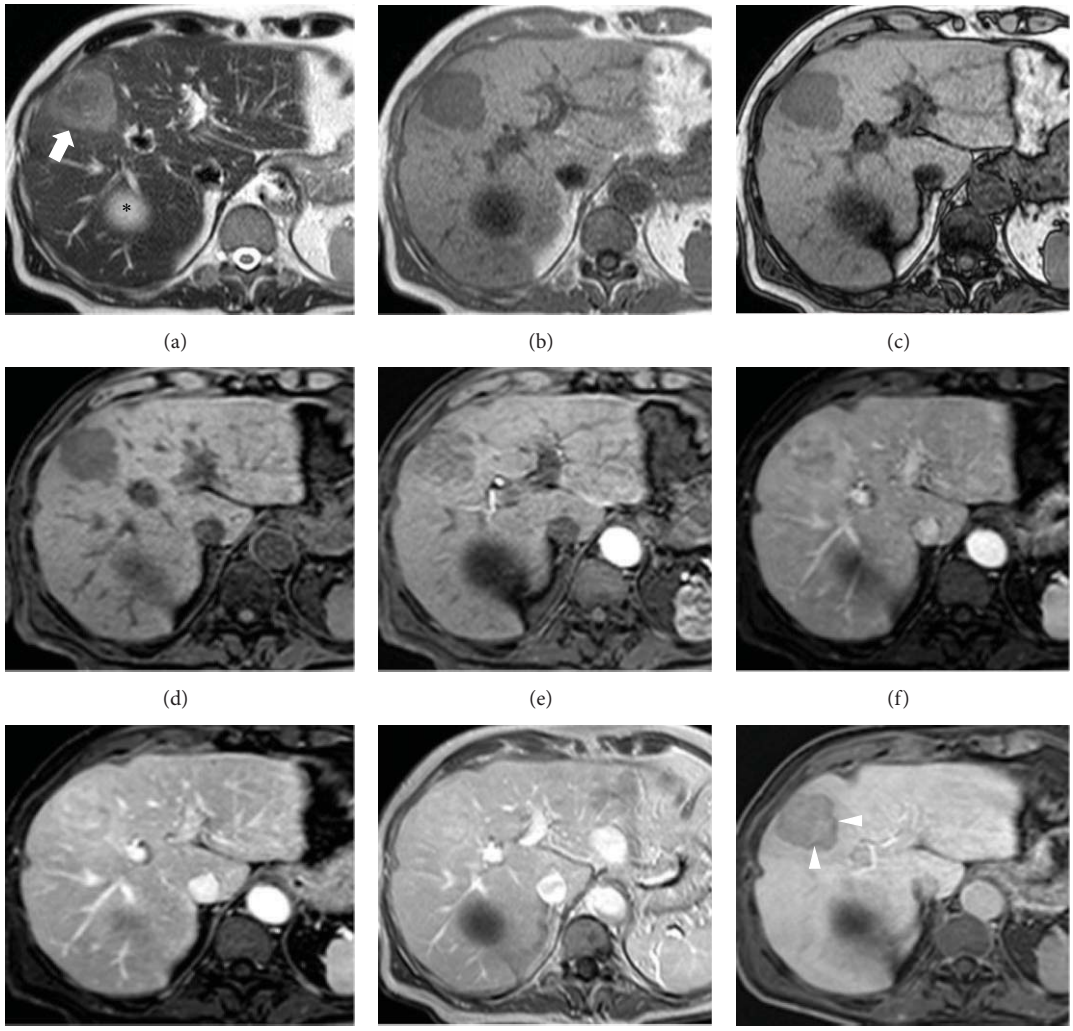

Figure 11: Peripheral CCC. (a) Axial T2w image. In S IV slightly heterogeneous hyperintense lobulated lesion (arrow); cyst (asterisk). (b-c) T1w in- and out-phased images. The lesion appears hypointense. (d–h) In dynamic evaluation after GD-BOPTA administration the nodule appears slightly heterogeneous hypervascular in arterial phase (e) with progressive enhancement in portal and equilibrium phases. After 5′ (h) note persistent enhancement and peripheral wash out. (i) Epatobiliary phase image after 1 h. The lesion appears hypointense, showing central pooling and more evident peripheral wash out (arrowheads).

and hypointense on T1-weighted sequences; they may show intense and heterogeneous enhancement in the arterial phase of dynamic study, subsequent wash out, and/or hypointense signal in hepatobiliary phase. The larger component of fat is more typical for HNF-1α-mutated HCA.

FL-HCC is usually either hypointense or, rarely, isointense compared to the liver on T1-weighted images. On T2-weighted images, 90% of the lesions are hyperintense and the remaining 10% are isointense. The purely fibrous nature of the scar means that it is hypointense on both T1- and T2-weighted images. FL-HCC becomes heterogeneously hyperintense during the arterial phase after the administration of gadolinium and appears as isointense or slightly hypointense during the portal-venous and equilibrium phase. The central scar shows minimal or no enhancement in arterial and portal-venous phase images but may sometimes show persistent enhancement in equilibrium phase. In hepatobiliary phases, FL-HCC usually appears as heterogeneously hypointense with areas of low signal intensity due to necrosis or, less frequently, haemorrhage (Figure 10) [42–44].

Besides the key elements already described for HCC in non cirrhotic patients, FL-HCC may be differentiated from HCA for areas of fibrotic tissue which are hypointense on T1 and T2w images and hyperintense in the delayed phases of the dynamic studies. Signs of malignancy (vascular and biliary invasion) present in HCC and FL-HCC are other helpful elements in differential diagnosis.

4.4. Cholangiocarcinoma. Cholangiocarcinoma (CC) is the primary malignancy arising from the bile duct epithelium and it is the second most common liver malignancy after HCC.

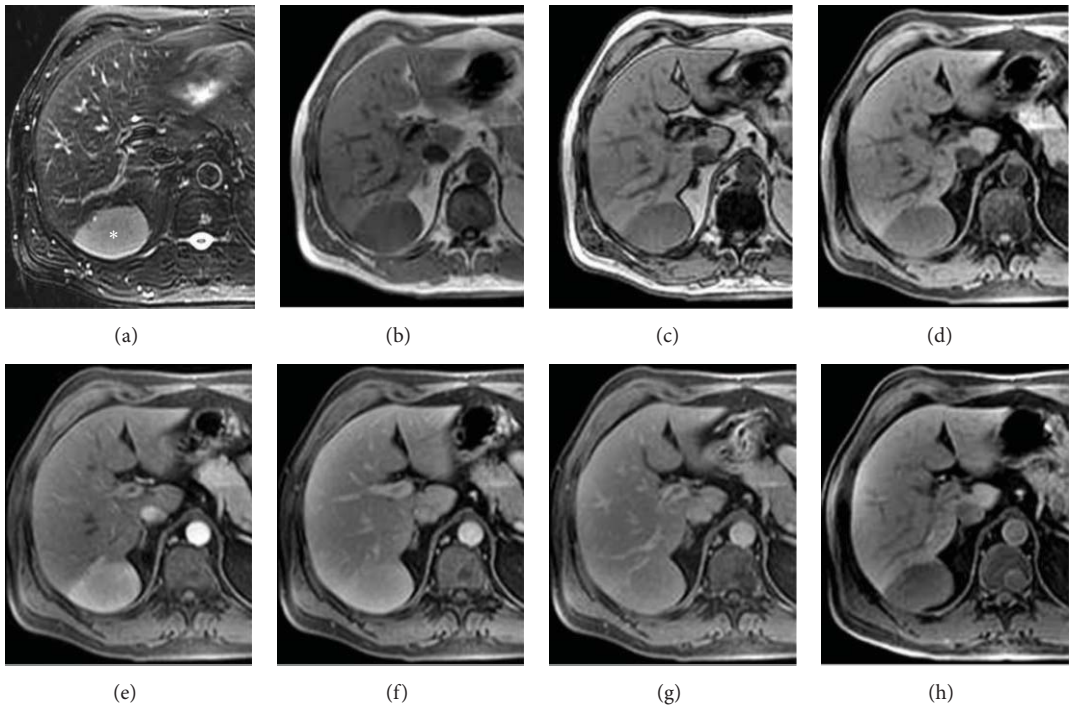

Figure 12: Primary hepatic NH lymphoma. (a) On T2w image well-defined homogeneous moderate hyperintense nodule (asterisk) in S VII. (b-c) T1w in- and out-phased images. The lesion appears homogeneously moderate hypointense on T1w sequences. (d–g) On contrast enhanced MRI the mass shows homogeneous hypervascular enhancement in arterial phase (e) and becomes isointense to liver parenchyma on portal and late dynamic phases. (h) In epatobiliary phase the nodule is homogeneously hypointense.

Incidence of CC among primary liver tumor ranges from 5 to 30%, with an average age of 50–60 years, seen slightly more often in men (M : F = 3 : 2). Risk factors are primary sclerosing cholangitis, bile stasis, recurrent cholangitis, infections with *Clonorchis sinensis* and *Opisthorchis viverrini*, hepatolithiasis, congenital bile ducts anomalies, familiar polyposis, Thorotrast deposition, and Caroli's syndrome. Recent findings indicate that HCV-HBV infection conferred a more than twofold elevated risk of ICC.

According to the site of origin CC can be classified into two types as follows: intrahepatic or peripheral (PCC) and extra-hepatic.

PCC presents as large mass because tumor does not cause clinical symptoms in early stages. The initial symptoms are abdominal pain, malaise, anorexia, weight loss, fever, palpable mass, and jaundice (rare).

The peripheral mass appears as a large white-grey lesion characterized by fibrosis (fibrotic core) and associated with capsular retraction; calcifications are rare. Sometimes there is concomitant dilatation of adjacent bile ducts and atrophy of corresponding liver segments.

Lymph node involvement is present in 60–70% of PCC.

Metastatic spread is common: lung, bone, pancreas, adrenals, kidney, spleen, and peritoneum.

According to the Liver Cancer Study Group of Japan (LCSGJ) and based on the gross appearance ICC can be categorized in three patterns of growth that can be present alone or in to combination: mass-forming type (MF), intraductal growth (IG) type, and periductal infiltrating type (PI). The prognostic significance of such classification has been confirmed in several clinical studies [45, 46].

Curative resection is the most effective treatment and the only therapy associated with prolonged disease-free survival; the rate of radical resection is extremely variable in the literature, ranging between 30% and 80%. Prognosis of ICC is poor due to late presentation and limited resectability. Five-year survival is less than 30% of surgically treated patients.

On MR imaging PCC is either isointense or hypointense relative to the normal liver on T1w MR images but may range from mildly to markedly hyperintense on T2w images. The signal intensity of the tumour is variable and depends on the amount of mucinous material, fibrous tissue, haemorrhage, and necrosis within the lesion.

On dynamic study after injection of Gd-BOPTA or Gd-EOB-DTPA, minimal or moderate incomplete enhancement is seen at the periphery on early images, whereas progressive central contrast enhancement is seen on later images. Generally, on delayed phase images lesions show peripheral hypointensity and central iso- or hyperintensity due to central pooling of contrast medium within central desmoplastic reaction. Satellite nodules are seen in about 10–20% of PCC cases and are chiefly responsible for the poor prognosis of this tumour (Figure 11) [47, 48]. The above three patterns are the key elements for the differential diagnosis with HCA.

4.5. Primary Lymphoma. Primary lymphoma (PL) of the liver (confined to the liver without involvement of lymph

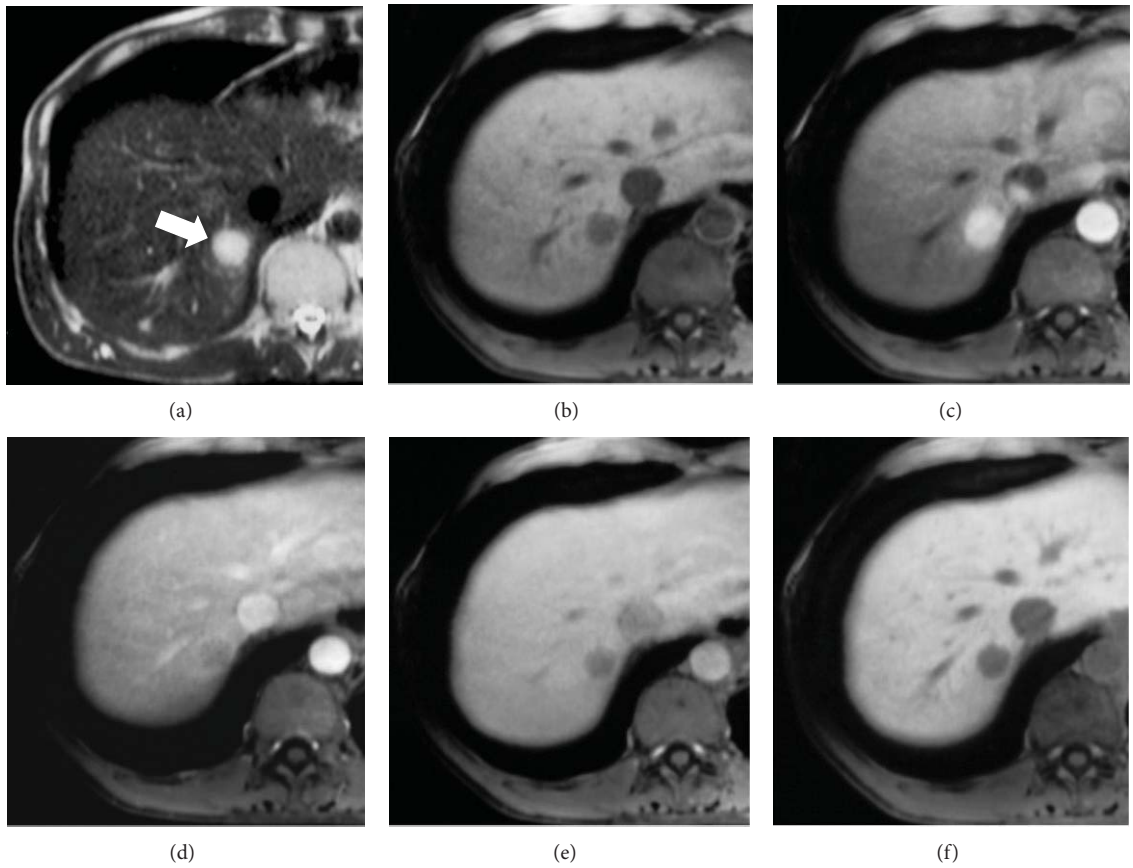

Figure 13: Neuroendocrine hepatic metastasis. (a) On T2w image well-defined homogeneous markedly hyperintense nodule (arrow) in S VII. (b–e) On precontrast T1w fat sat images the lesion is hypointense (b). On contrast enhanced MRI the nodule shows homogeneous evident enhancement in arterial phase (c) with rapid wash out in portal phase, more evident in equilibrium phase. (f) In epatobiliary phase, 10' after GD-EOB-DTPA, the nodule is hypointense.

nodes or spleen or bone marrow) is very rare (0.4% of extranodal non-Hodgkin's lymphomas and 0.2% of all non-Hodgkin's lymphomas). PL can arise at every age but it is more frequent in childhood-adolescence or in middle-old age (M : F = 4 : 1). It can be associated with Epstein-Barr infection (EBV), and more frequently in patients with chronic hepatitis or cirrhosis (by HBV and/or HCV infections), with autoimmune disorders or with AIDS manifestations; it can also be associated with immunosuppression in transplanted patients. Liver involvement (50% of cases) in haematological diseases (secondary liver lymphoma) is more common.

Hepatomegaly, presence of hepatic mass or masses, and pain in upper right quadrant are the most frequent signs and symptoms in primary lymphoma. Fever, weight loss, and perspiration are associated in 50% of cases.

PL can present as multiple, small nodular lesions (well-differentiated B-cell lymphoma) or as diffuse infiltration of hepatic parenchyma (undifferentiated subtypes); in some patients with Hodgkin's lymphoma peliosis hepatis can be associated.

The outcome, with appropriate treatment, is favourable. Surgical resection followed by chemotherapy and/or radiation gives the best prognosis in primary lymphoma [49, 50].

On MR imaging, PL is generally seen as homogenously/heterogeneously hypointense compared to the normal parenchyma on unenhanced T1-weighted images and hyperintense on T2-weigthed images. Dynamic imaging after Gd-BOPTA or Gd-EOB-DTPA typically reveals a hypointense appearance on arterial phase images, followed by homogeneous, delayed enhancement on portal-venous phase images, and isointensity on equilibrium phase images. However, some hypervascular PLs are described. In most cases, PL is hypointense in the delayed hepatobiliary phases (Figure 12) [51].

The equivocal behavior of PL does not offer important elements for differential diagnosis with HCA. However, HNF-1α-mutated HCA may be differentiated for the fatty intralesional component which determines signal patterns on plain MR and dynamic study.

4.6. Metastases. Metastases are the most common cause of malignant focal liver lesions (18–20 times more frequent than primary malignant tumors). At autopsy hepatic metastases occur in 30 to 55% of patients dying from malignant disease. Liver metastases originate predominantly from primary tumors localized in gastrointestinal tract (colon, stomach,

Table 1: Hypervascular lesions in noncirrhotic patients. The most important elements to achieve the differential diagnosis.

		MRI features									
	Risk factor and clinical setting	T2w	T1w in	T1w out	DWI	Pre-c	ArtP	PVP	EqP	HBP	Specific pattern
Inflammatory adenoma		Hyper	Hypo	Hypo	Restriction	Hypo	Hyper +	Hyper	Hyper/iso	Hypo/hyper	Signal heterogeneity, hypervascularity, intralesional hemorrhage
HNF-1α-mutated adenoma	Obesity, steatosis, diabetes, glycogenesis, exogenous steroids	Iso/hyper	Hyper	Hypo	No restriction	Iso/hypo	Hyper	Hypo	Hypo	Hypo	Fatty intralesional component
β-catenin-mutated adenoma		Hyper	Hypo	Hypo	Restriction	Hypo	Hyper +	Hyper	Hyper/iso	Hypo/hyper	Intralesional hemorrhage, necrosis
Unclassified adenoma											Nonspecific patterns reported
Focal nodular hyperplasia	Exogenous steroids	Iso	Iso	Iso	No restriction	Iso	Hyper +++	Iso	Iso	Iso/hyper	Signal homogeneity, hypervascularity, scar, iso/hyperintensity on HbP
Large regenerative nodule	Myeloproliferative and lymphoproliferative disorders, chronic vascular and rheumatologic syndromes	Iso	Iso	Iso	No restriction	Iso	Hyper ++	Iso	Iso	Iso/hyper	Multiple lesions signal homogeneity, hypervascularity, scar, isohyperintensity on HbP
HCC in noncirrhotic liver and FL-HCC	Exogenous steroidenvironmental and dietary factors (aflatoxins and nitrosamine)	Hyper	Hypo	Hypo	Restriction	Hypo	Hyper ++	Hypo	Hypo	Hypo	Signal heterogeneity, intralesional hemorrhage, signs of malignancy
Cholangiocarcinoma	Viral hepatic infection	Hyper	Hypo	Hypo	Restriction	Hypo	Hyper	Hypo	Hypo/iso	Hypo/hyper	Peripheral enhancement, peripheral washout, central pooling, signs of malignancy
Primary lymphoma	Epstein-Barr infection (EBV), HBV and/or HCV infections	Iso/hyper	Hypo	Hypo	Restriction	Hypo	Hyper	Hypo	Hypo	Hypo	Variable pattern, small lesions hypervascular, significant restriction
Metastases	Primary tumor, metastatic syndrome	Hyper	Hypo	Hypo	Restriction	Hypo	Hyper ++	Iso/hypo	Hypo	Hypo	Multiple lesions hypervascularity, halo signs signs of malignancy

and pancreas in decreasing order of frequency), by hematogenous spread, via portal vein. Other frequent secondary lesions are from breast and lung cancers (but also endocrine/neuroendocrine tumors and melanoma): primary neoplasms probably give metastases by hematogenous spread, via the arterial blood supply to the liver. Lymphogenous spread occurs along bile ducts from near organs (pancreas) or from haematological diseases (lymphoma, leukaemia). Direct invasion from nearest organs (gallbladder or bile duct cancer, pancreatic neoplasms) can also be the cause of liver metastases. Liver involvement by intra-abdominal dissemination can also be observed (ovarian cancer) [52].

Most patients with metastases to the liver present with symptoms related only to the primary tumor; the asymptomatic hepatic involvement is discovered in the course of clinical evaluation. Sometimes there are no specific symptoms: weakness, weight loss, fever, and loss of appetite. Rarely, hepatomegaly, hepatic mass, or pain in upper right quadrant can be the first symptoms of liver involvement mostly when lesion/s are huge and multiple.

Metastases can vary in size, number, consistency, uniformity of growth, vascularity, and stromal response.

The outcome depends not only on primitive tumor, but even on number, size, and tissue component of metastases. If resectable, hepatic metastases have 20–40% survival rate at 5 years (colon cancer metastases).

The differential diagnostic dilemma between HCA and metastases is mainly in the case of hypervascular, solitary lesion in patients with unknown primary cancer.

Generally, on unenhanced T1w images metastases have low signal intensity compared to the surrounding parenchyma. On T2w sequences the lesions demonstrate high signal intensity, although the signal is generally lower than that typically observed in hemangiomas. Hypervascular metastases tend to reveal strong transient enhancement in the arterial phase followed by hypointensity in the portal and equilibrium phases; they appear hypointense in hepatobiliary phase (Figure 13) [41, 53, 54].

The most important elements to achieve the differential diagnosis between HCA and each type of hypervascular lesions in non cirrhotic patients are summarized in Table 1.

5. Conclusions

According to recent studies HCAs are currently categorized in to four distinct genetic and pathologic subtypes: inflammatory hepatocellular adenomas, hepatocyte-nuclear-factor-1-alpha (HNF-1α-mutated) hepatocellular adenomas, β-catenin-mutated hepatocellular adenomas, and unclassified adenomas. This classification has definitive management implications. MR imaging plays an important role in diagnosis and characterization, particularly in inflammatory and steatotic subtypes, as well as in identification of complications and surveillance.

Image-guided biopsy or surgical resection with histopathologic and immunohistochemical analysis is necessary for complete characterization of HCAs and in some differential diagnostic dilemmas.

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Review Article

Benign Hepatocellular Tumors in Children: Focal Nodular Hyperplasia and Hepatocellular Adenoma

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Benign liver tumors are very rare in children. Most focal nodular hyperplasia (FNH) remain sporadic, but predisposing factors exist, as follows: long-term cancer survivor (with an increasing incidence), portal deprivation in congenital or surgical portosystemic shunt. The aspect is atypical on imaging in two-thirds of cases. Biopsy of the tumor and the nontumoral liver is then required. Surgical resection will be discussed in the case of large tumors with or without symptoms. In the case of associated vascular disorder with portal deprivation, restoration of the portal flow will be discussed in the hope of seeing the involution of FNH. HepatoCellular Adenoma (HCA) is frequently associated with predisposing factors such as GSD type I and III, Fanconi anemia especially if androgen therapy is administered, CPSS, and SPSS. Adenomatosis has been reported in germline mutation of HNF1-α. Management will depend on the presence of a predisposing factor and may include metabolic control, androgen therapy withdrawn, or closure of the shunt when appropriate. Surgery is usually performed on large lesions. In the case of adenomatosis or multiple lesions, surgery will be adapted. Close followup is required in all cases.

1. Introduction

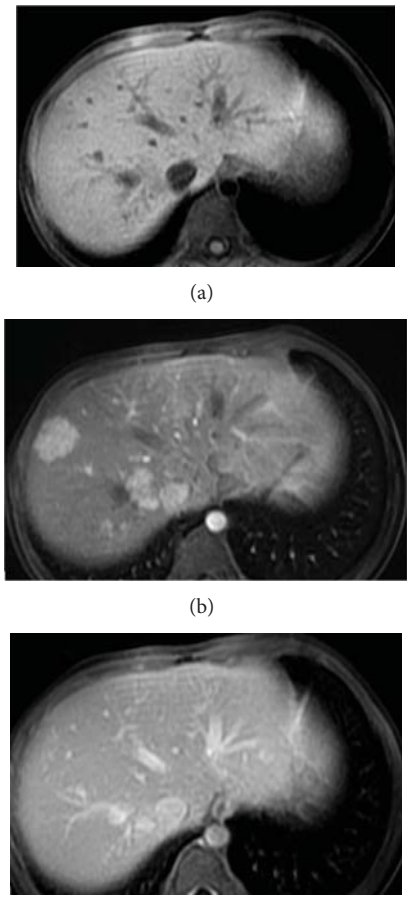
Liver tumors are very rare in children, accounting for 1 to 4% of all pediatric tumors. Benign tumors account for 30 to 40% of these, with a majority of hemangiomas occurring during infancy. Focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCAs) are extremely rare during childhood, and there are few published reported cases and series. Presentation, physiopathology, and management differ from adults.

We will successively review the main characteristics of FNH and HCA in children and discuss physiopathology, followup, and therapeutic modalities based on a systematic review of the literature and our experience.

2. Focal Nodular Hyperplasia

2.1. Histological Definition and Physiopathology. Focal nodular hyperplasia (FNH) is not a neoplasm but a nonspecific hyperplasic reaction to vascular abnormalities. It is a well-delimitated lesion without capsules and characterized by hepatocytic nodules separated by fibrous bands. The mass has a central stellate fibrous region containing malformed vascular structures that include large arteries, without portal veins. Bile ductular reaction is usually present at the interface between hepatocytes and fibrous bands and is highly suggestive of the diagnosis of FNH. According to some authors, in FNH, arterial blood flows from the anomalous arteries via capillaries into sinusoids adjacent to the fibrous septa. The blood in the sinusoids drains to the hepatic vein either directly or via perinodular veins. The absence of portal vein branches in FNH leads to the absence of portal blood flow.

The precise cause of FNH is unknown. Several theories have been suggested to explain the occurrence of FNH: vascularization by an anomalous large artery, acquired thrombosis, reactive hyperplasia after hepatocellular injury induced by



(a)

(b)

(c)

Figure 1: Nine-year-old girl with a history of metastatic right nephroblastoma treated with chemo- and radiotherapy. Liver MRI with T1-weighted images without (a) and with contrast injection at the arterial (b) and portal phases (c), performed six years after the end of treatment, displays multiple hepatic nodules, from 1 to 3 cm large, that enhance strongly after contrast injection at the arterial phase (b) and have almost the same signal as the surrounding liver before contrast injection (a) and at the portal phase (c). Simple clinical and imaging followup was performed (courtesy of Dr. H. Brisse, Institut Curie Paris, France).

vasculitis, or higher blood flow, either portal or arterial, compared with the surrounding tissues [1–8].

2.2. Frequency and Predisposing Factors in the Pediatric Population. FNH is very uncommon in children. About 200 cases have been reported in the literature, with few short series [9–14]. It represents from 2% to 7% of pediatric liver tumors [10, 13, 15]. FNH has been reported in all pediatric age groups, including prenatal and neonatal forms [16–18].

First known as an incidental lesion, FNH can also be associated with predisposing factors such as chemotherapy and radiation therapy in children treated for malignancy, and portal deprivation in case of congenital or surgical portosystemic shunts (CPSSs, SPSSs) (Figures 1 and 2).

In the group of children with no predisposing factors, the incidence is estimated to be 0.5%. There is a female predominance as in adulthood. Mean age at diagnosis is between eight and 11 years [11].

In the population of long-term survivors of pediatric malignancy, the incidence of FNH is higher than in the general population and has been estimated to be 5%. This represents about one-third of children with FNH, but the number of cases reported is increasing as survivorship has significantly improved in the past decades. There is a male predominance, and mean age at diagnosis is older, between 10 and 16 years. Most patients have a history of malignancy or hematologic disorder requiring stem cell or bone marrow transplant (BMT). High doses of alkylating agents (busulfan and/or melphalan) that are very hepatotoxic and incriminated in hepatic venoocclusive disease and radiotherapy have been reported to be a risk factor for FNH. The mean time to develop FNH after treatment has been estimated to be between four and 12 years (from two to 27 years). This delay was shorter in children who had undergone high-dose chemotherapy along with BMT (7 years) (range 3–10 years). This delay was around 12 years (range 2–20 years) for patients who had not received this type of chemotherapy [9, 13, 19–21].

FNH has also been reported in children with congenital or surgical portosystemic shunt (CPSS and SPSS) and is probably secondary to complete or partial diversion of portal blood through the shunt, which leads to impaired portal blood supply with hyperarterialization of whole or part of the liver parenchyma [22, 23].

2.3. Clinical Presentation. Symptomatic FNH are more frequent in children than in adults and are found in about one-third of the patients. Two-thirds of the patients with tumors larger than 7 cm are symptomatic [11]. The most frequent symptom is abdominal pain. More rarely, weight loss and weakness can be encountered, mostly in very large tumors, and these symptoms can give concern for malignancy.

Patients who have a history of malignancy are more likely to have small asymptomatic lesions discovered on routine surveillance (Figure 1) [13, 21]. FNH associated with CPSS can be discovered either during routine surveillance of the vascular malformation or may be fortuitously discovered and then reveal the vascular malformation [22].

2.4. Imaging Features. FNH appears on US as a well-delimitated, lobulated mass that is iso- or slightly hypo- or hyperechoic compared to the surrounding liver. On Doppler examination, it is usually fed by a large artery with a stellate structure of its branches within the tumor. Typical radiologic findings on imaging techniques using contrast enhancement include a solitary, homogeneous, and slightly hypoattenuating mass compared to the surrounding liver on unenhanced CT with rapid homogeneous contrast enhancement at the arterial phase (except for the central scar). On venous phase, the mass becomes isodense as compared to the surrounding liver, while the central scar might be enhanced on the late phase. On MRI, FNH is usually hypo- or isointense to the surrounding liver on T1-weighted images and iso- to slightly

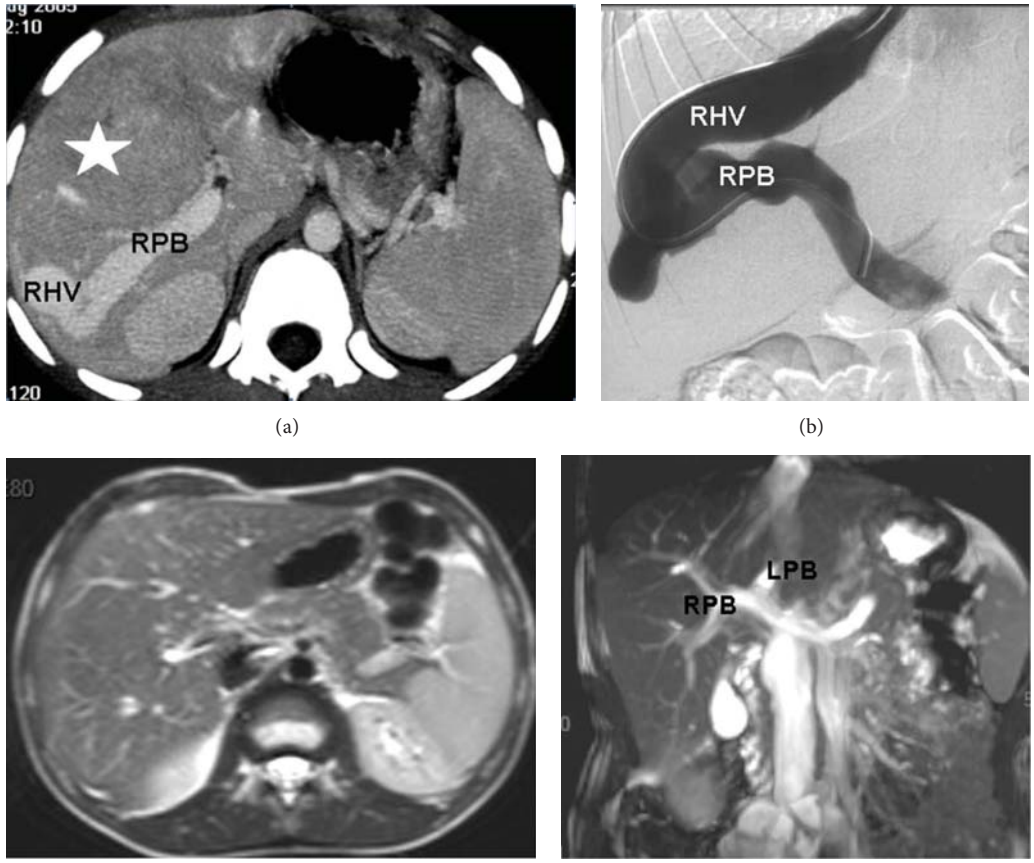


Figure 2: Six-year-old boy with a congenital portohepatic shunt complicated by a biopsy-proven FNH measuring 7 cm diameter (white star). (a) Contrast-enhanced CT scan at diagnosis shows an abnormal and large communication between the right portal branch (RPB) and the right hepatic vein (RHV). (b) Phlebography with opacification of the shunt between the RPB and the RHV. Closure of the shunt was performed by interventional radiology. (c) MRI performed seven years later shows the disappearance of FNH on the T2-weighted images. No enhancement was present at the arterial phase after gadolinium injection (not shown). (d) Note the normal aspect of the portal bifurcation (RPB and left portal branch (LPB)) on coronal MIP reconstruction of the T2-balanced sequence.

hyperintense on T2-weighted images. Enhancement after gadolinium injection is similar to that observed on a CT scan [20, 24]. There is no calcification. In adults, due to the high specificity of CT and MRI in diagnosing FNH, there is usually no indication for biopsy in the presence of typical radiological features. In children, there is no study validating these criteria, but in our experience, when typical features of FNH are present, biopsy is not mandatory to confirm diagnosis.

The diagnosis of FNH in children can be challenging as atypical lesions occur in about two-thirds of cases, and multiple lesions are more common in children than in adults [25]. Imaging features will depend on the context. In patients with no predisposing factor, FNH are frequently larger in children than in adults (64% of tumors >5 cm versus 20% in adults [9–11, 15, 26–29]. In most cases, arterial strong enhancement of the lesions on contrast-enhanced CT or MRI is present. It can be absent if there is a complete portal diversion secondary to CPSS or SPSS, as the surrounding liver may also be fed only by the hepatic artery. A central scar is very rare in the case of small or multiple FNH. It is better seen on MRI when present [11]. Fibrolamellar hepatocarcinoma is an important differential diagnosis as it frequently presents with an area of scarring. Patterns that are in favor of fibrolamellar hepatocarcinoma are lobulated margins of the tumor, the presence of calcifications, the large size of the central scar, the tumor heterogeneity before injection and at the arterial phase, and the presence of lymphadenopathies and/or the presence of metastases [30].

In the group of children with a history of chemo- and/or radiotherapy, a major concern is to differentiate benign and malignant processes. Strong enhancement at the arterial or early portal phase is important to differentiate FNH or "FNH-like" lesions from metastases that usually remain hypointense on arterial or early portal phase when compared to the surrounding liver [21]. Even if not presenting the typical diagnostic criteria for FNH, multiple liver lesions strongly enhancing at the arterial phase after injection in a long-term cancer survivor are highly suggestive of the diagnosis, and

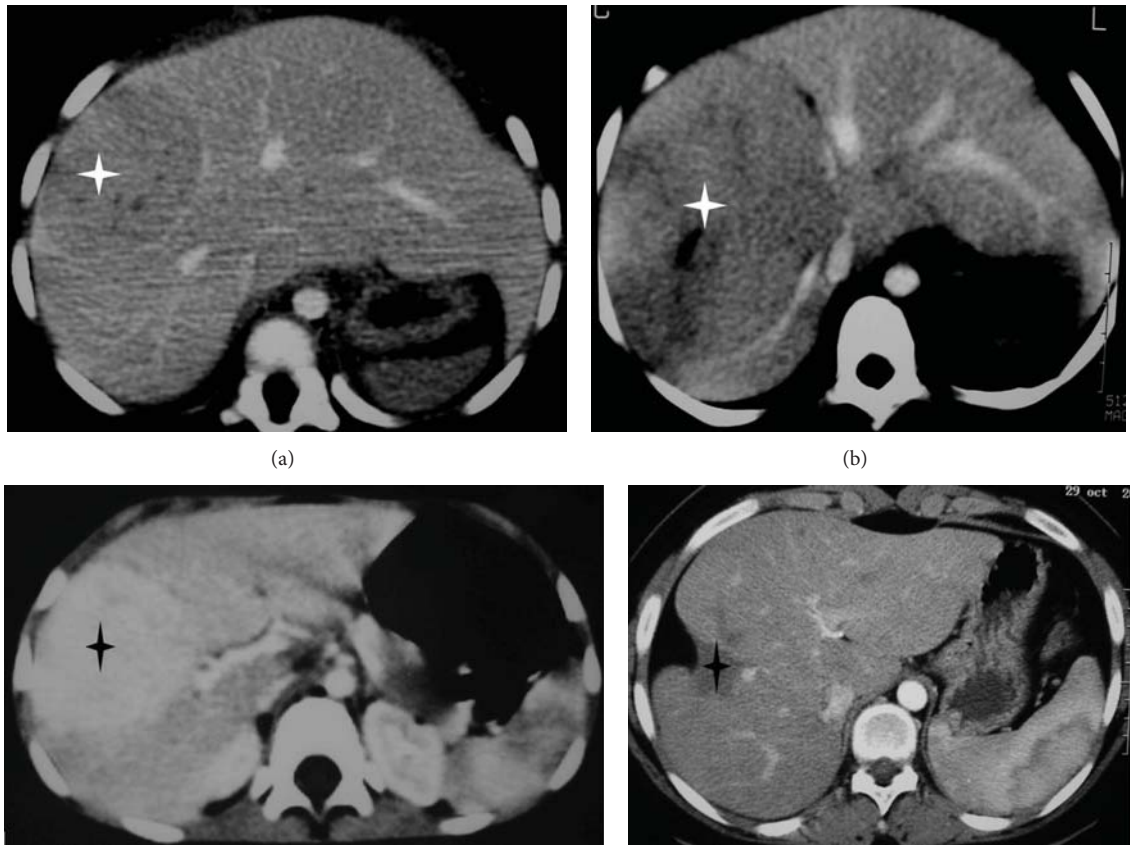


Figure 3: Spontaneous evolution of fortuitously discovered FNH in two children. (a) and (b): three-year-old girl with sickle-cell disease. CT scan after contrast injection at the portal phase at diagnosis (a). And three years later (b) shows growth of the tumor from 5 to 12 cm diameter. (c) and (d): eight-year-old girl. CT scan after contrast injection at diagnosis (c) and 12 years later (d) shows the spontaneous disappearance of the 5 cm diameter tumor with capsular retraction.

conservative treatment with imaging surveillance should be recommended (Figure 1) [11, 13, 21, 31].

2.5. Natural History and Management. In the literature, few patients had conservative management of FNH. The natural history of these tumors is poorly known in children. All kinds of spontaneous evolution have been described, from spontaneous involution to growth (Figure 3) [11].

In the literature, 78% of the patients with no history of malignancy or hemopathy had a surgical resection. Most masses were large [11, 12].

In our practice, when the imaging features are typical on MR and/or CT, the patient is asymptomatic and there is no associated vascular disorder, biopsy is not performed, and conservative management is proposed with a prolonged followup by ultrasound to show if there is an increase in size.

When the aspect is not typical, biopsies of the mass and the nontumoral liver are performed to assess the diagnosis of the tumor and search for associated abnormalities of the liver that could be part of an unknown predisposing factor for a tumor.

Surgery is sometimes performed in the case of very large lesions, symptoms, and/or impairment of physical activities. Recurrence of FHN after surgery has been reported in one case in the literature, and we have a personal case, not published [32].

FNH secondary to CPSS requires special management as the closure of the shunt with restoration of intrahepatic portal flow may lead to shrinkage of the tumor, as shown in previous cases (Figure 2). That is why, whatever are the size of the tumor, the number of lesions, and their location, closure of the shunt should be performed when possible. We usually perform a biopsy of the tumor and the nontumoral liver to confirm the diagnosis of benign liver proliferation and exclude a hepatoportal sclerosis that would contraindicate the closure of the shunt [22].

In the case of SPSS, closure of the shunt should be discussed in regard to the patient's history. Restoration of intrahepatic portal flow by making a mesenterico-rex bypass should be performed when possible, mostly in the case of portal obstruction with cavernomatous transformation [23].

3. Hepatocellular Adenoma

3.1. Histopathological Definition. Hepatocellular adenomas (HCAs) are extremely rare during childhood. They are benign

liver tumors that represent a heterogeneous group in which histopathological features may vary according to the etiological background. Classically, HCAs are soft, well-demarcated tumors with little or no fibrous capsule. They are composed of liver cell plate mildly thickened or irregular. They are supplied by thin-walled arteries without other portal tract elements (connective tissue, bile ducts, or ductular reaction).

In adults, genomic and molecular studies together with the analysis of genotype/phenotype correlations have led to the recognition of four major HCA subgroups: HNF1-α-inactivated HCA, β-catenin-activated HCA, and two forms of HCA without mutation of HNF1-α or β-catenin presenting either with or without inflammation. These different subtypes display variable clinical behavior, imaging findings, and natural history that have recently been well described [33–36]. To our knowledge, the only study concerning the profile of HCA genotype-phenotype in children concerns glycogen storage disease type I (GSD) and showed a high frequency of β-catenin mutations and lack of HNF 1α inactivation [37].

HCA formation is complex and varies according to the etiological background. Natural history and management vary with the context.

3.2. Epidemiology and Predisposing Factors in Children. According to the rare published pediatric series, HCA occurs in 0 to 21% of pediatric benign liver tumors. Differences in the frequency between series are probably related to the differences in patients' recruitments. The largest pediatric series reported 22 HCA in a 12-year period [12, 38–41].

Most HCAs are diagnosed during the teenage years (the mean age at diagnosis is around 14 years). HCAs reported before the age of one year are exceptional, the youngest patient being three weeks old in a context of multiple congenital anomalies [42].

Sex ratio varies with series, but the female predominance observed in adults is not the rule in children, and male predominance is observed in several series [38].

During childhood, HCA can be sporadic but is more frequently associated with predisposing factors such as GSD type I and III, anabolic androgenic steroid treatments with or without Fanconi anemia, congenital or surgical portosystemic shunt (CPSS, CPSS), germline mutation of HNF1-α gene, and familial adenomatosis polyposis (Figures 4, 5, and 6) [22, 23]. Hurler syndrome, Turcot syndrome, Lynch syndrome, immunodeficiency syndrome, tyrosinemia, and galactosemia have also been reported, and we have the personal unpublished experience of teenage girls with multiple HCA associated with Glanzmann's thrombasthenia treated by progestative therapy [43].

3.3. Imaging Features. Making the diagnosis of HCA by imaging can be challenging. In adults, major improvements in knowledge of HCA have been gained during recent years. Correlations of imaging with the genotype/phenotype classification proposed by the "Bordeaux" experience have made it possible to distinguish specific imaging patterns in relation with the two major subtypes, inflammatory HCA and HNF 1α-mutated HCA that account for 80% of all cases in adult series [34, 35, 44]. Very little data on the imaging features of HCA are available for children.

Ultrasonography is usually the first exam performed in children for the evaluation of abdominal disorders and the first screening tool during followup in predisposed children, but it can miss small nodules isoechoic to the liver, especially in a steatotic liver. HCAs are typically heterogeneous and are well-delimited solid masses with vessels within the mass (Figure 5).

Magnetic resonance imaging (MRI) is the best technique to depict lesions. HCAs patterns on MRI will depend on the amount of fat, hemorrhage, and necrosis within the mass. HCA are frequently heterogeneous on T1- and T2-weighted images (WIs) but with a high signal on T2 WI. Fat component in HCA is well demonstrated with chemical shift sequences as in "in-phase and out-of-phase" T1 sequence. It can also be shown by sequences with fat saturations that are less sensitive. When present, it can help diagnosis. Early enhancement after contrast injection is observed in most cases. An enhanced pseudocapsule can be visible on delayed acquisition. Washout should not be too early (Figure 4). Diffusion sequences may help in the detection of small lesions. CT can also display fat within the tumor, heterogeneous content and early arterial enhancement [45]. In the presence of CPSS or SPSS, both HCA and nontumoral liver will lack portal vascularization, causing the absence of the early arterial enhancement classically observed in HCA, as both the nodule and the surrounding liver only have arterial supplies.

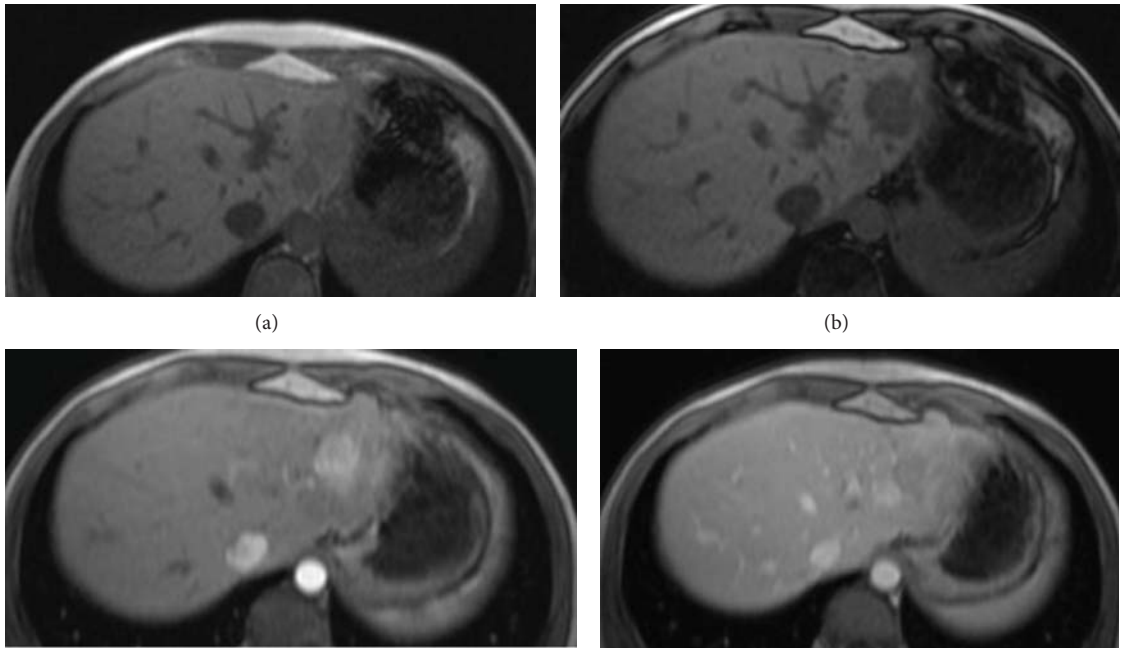
When sporadic, HCA are frequently large solitary masses. In predisposed children, multiple HCA are more frequently observed. The term "adenomatosis" was first used in adult literature, and its definition excluded patients with GSD and steroid treatment [46]. This term has also been used for adenomas related to HNF1-α mutation (Figure 4).

Except for children with GSD, there are no published recommendations about screening protocols for HCA in patients presenting predisposing factors. At least annual ultrasonography should be performed, but MRI with its better sensitivity for the detection of liver tumors mostly in older children could be part of the systematic screening using T1-WI with fat suppression or chemical-shift sequences and T2-WI, diffusion sequences, and dynamic gadolinium injection when a lesion is detected on initial sequences.

An important concern is the detection of malignant transformation. This may be challenging and only possible with histology. Increasing size of the tumor and modifications of its aspect can be signs of malignant transformation. The kinetics of enhancement after contrast injection are important, and early washout after early enhancement at the arterial phase is suggestive of HCC transformation.

In most cases, histologic assessment of the tumor is necessary to adapt the management. In sporadic cases with no known predisposing factor, it is worthwhile to perform a biopsy on the nontumoral liver in order to depict an unknown underlying liver disease.

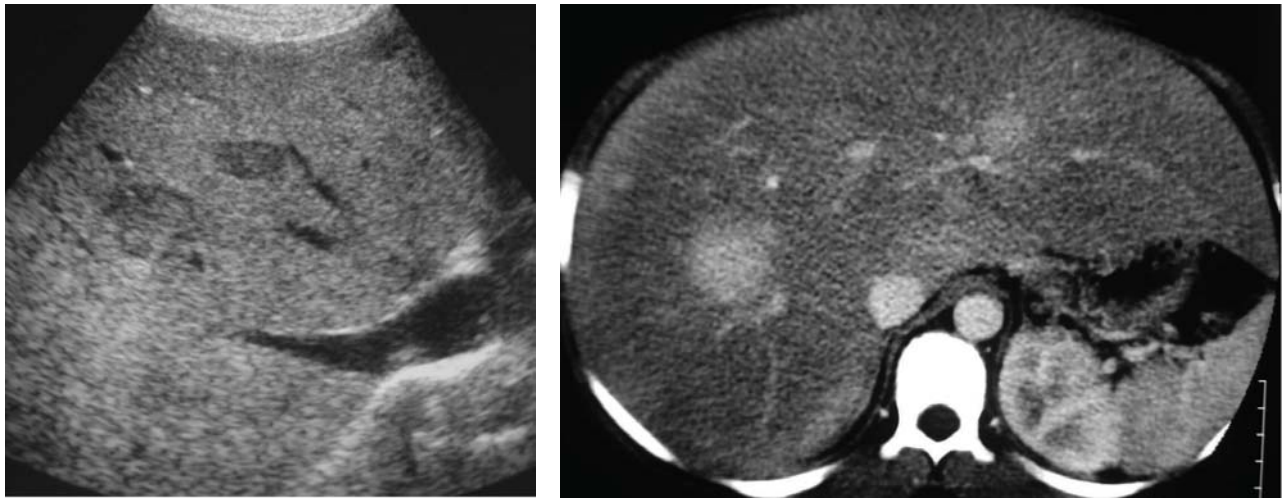
3.4. Natural History and Predisposing Factors. In sporadic cases or when no predisposing factor is known, HCA can be



(a) (b)

(c) (d)

Figure 4: Adenomatosis related to HNF1-α germline mutation aspect on MRI in a 14-year-old girl: (a) and (b): T1 WI with chemical shift shows two lesions in the left lobe of the liver with drop of the signal of the largest lesion on the out-phase sequence that reveals the presence of fat in the tumor. (c) and (d): T1 WI after contrast injection shows early arterial enhancement of the largest lesion with washout in the late portal phase.



(a) (b)

Figure 5: Fourteen-year-old boy with glycogen storage disease type I and multiple HCA measuring from 1 to 3 cm diameter. (a) US shows an enlarged hyperechoic liver (steatotic) with well-delimited hypoechoic nodules. (b) CT performed at the arterial phase after contrast injection shows enlarged steatotic liver with multiple nodules that are strongly enhanced.

found incidentally during imaging for unrelated pathology, but most often patients present with abdominal pain or palpable mass to the right upper quadrant or the epigastric region.

The two major concerns with HCA are hemorrhage and malignant transformation into HCC.

Hemorrhage is one of the most important complications of HCA. The maximum risks of bleeding and rupture have been estimated in 27.2 and 17.5 percent of patients respectively in a systematic review with the youngest patient being 14 years old [47]. Two types of hemorrhagic changes can take place in HCA: internal hemorrhage usually mixed with

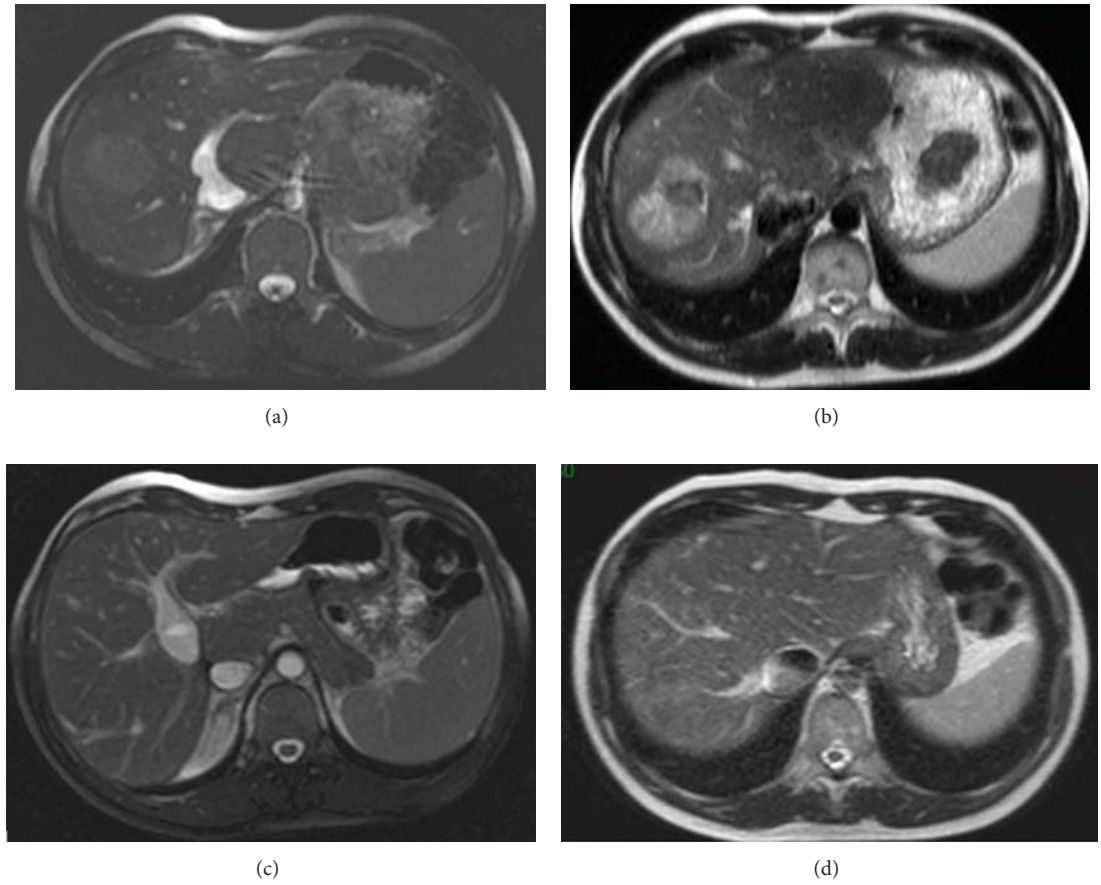


Figure 6: Adenoma associated with CPSS. Aspect on MRI at diagnosis and 10 months after surgical closure of the shunt: (a) T2 WI-balanced sequence shows the CPSS consisting of a patent ductus venosus. Note that the tumor is not easily visible in this sequence. (b) The 4 cm diameter HCA lies in segment 8, and it is better seen on T2 WI and appears heterogeneous and mainly hyperintense compared to the surrounding liver. MRI performed 10 months after surgical closure of the shunt (c) shows the complete disappearance of the tumor (d).

necrotic changes (usually in tumors larger than 4 cm) and spontaneous rupture with possible subcapsular hematoma and/or hemoperitoneum. Severe abdominal pain with possible hemodynamic disorders or even collapse can occur during intraperitoneal or intratumoral hemorrhage of HCA. Fatal issues have been reported in young patients with familial adenomatosis related to HNF1-α mutation and in FA. Hemorrhage has been reported in FA even after discontinuation of androgen therapy [48, 49].

Malignant transformation of HCA into HCC is rare, even in adults, and remains controversial in the literature [50]. Among the 50 cases (4.2%) of malignant transformation reported in a systematic review of 1635 HA (glycogenosis and adenomatosis were excluded), there were no pediatric cases [51]. In children, exceptional malignant transformations of HCA have been reported, mainly associated with GSD, FA with steroid therapy, and CPSS [22, 33, 51–55]. HCA associated with GSD type I displays high frequency of β-catenin mutation that could explain the high frequency of malignant transformation [37]. In adults, risk groups for malignant transformation of HCA are male gender, tumors larger than 5 cm, and β-catenin-activated HCA, and even though no data are yet published in the literature, these criteria should be taken into account when managing children. [51–53, 56].

In children with predisposing factors, the natural history and management of HCA will depend on the underlying pathology.

Concerning HCA complicating GSD type I and III, most series or cases are associated with type I. In a series of 43 patients with GSD, about 52% of patients with type I and 25% of patients with type III glycogen storage disease had HCA [57]. Natural history and pathophysiological conditions remain poorly understood. HCA develop predominantly during and after puberty, between the ages of 10 and 20 years, and the incidence of new HCA appears to decrease after 20 years of age. The youngest patient presenting with HCA was 3.6 years old [58]. The male to female ratio is 1/1. The increased incidence of HCA development during adolescence may be related to suboptimal metabolic control during this period and/or to pubertal hormone secretion. Metabolic control seems to be an important factor for HCA development. Considerable alteration of lipid metabolism is a feature encountered in GSD associated with HCA formation.

According to some authors, GSD type I displays a very high level of de novo fatty acid synthesis, which is known to play a role in tumorigenesis [58, 59].

Malignant transformation leading to HCC has been reported in adult patients and is probably related to the high frequency of β-catenin mutation [37, 52, 54, 55]. Recommendations for screening HCA by the "European Study of Glycogen Storage Disease Type I b" include an annual abdominal ultrasonography from birth to 10 years old, then every six months after 10 years of age.

On liver ultrasound, HCAs are usually well-delimited round nodules hypoechoic compared to steatosis surrounding the liver parenchyma. If liver HCA is detected, ultrasonography should be performed every three months, associated with dosage of serum α-foetoprotein and carcinoembryonic antigen. CT or MRI with contrast injection will be performed on demand if the nodules increase in size, or if features suggestive of malignant transformation appear [60]. However, the difficulty in detecting HCA in bright liver with attenuation of US beam makes MRI more reliable for screening in some patients.

Management of HCA includes metabolic control as, when metabolic control is achieved, regression of HCA has been reported [61]. Surgery will depend on the presentation, ranging from tumorectomy to liver transplantation.

In children with Fanconi Anemia (FA) and androgen therapy with or without FA, liver tumors can occur and concern about 3% of patients [62]. Most are HCAs, but hepatocellular carcinoma (HCC), focal nodular hyperplasia (FNH), hepatoblastoma have also been reported [63–65]. Treatment of FA is based on androgen therapy and BMT as in other forms of aplastic anemia. Liver tumors can occur in FA patients in the absence of androgen therapy but are mainly associated with it. FA patients usually start androgens when they are young, at a median age of 7.5 years. Median duration of treatment with androgen prior to HCA or HCC diagnosis is four years, and median age at diagnosis of HCA is 12 years [64]. Association of HCA and HCC has been reported in several patients, and there is more likely to be transition from HCA into HCC as suggested by the presence of foci of HCC within HCA [63]. The median age at diagnosis of HCC in FA is 13.4 years [64]. Several factors may play a role in the development of HCA in FA: (i) genetic disorders and chromosomal defects allow mutagenesis and liver cell proliferation, (ii) chronic iron overload, which is frequently encountered even in the absence of blood transfusion or hemochromatosis, probably has a carcinogenetic effect [66, 67], and (iii) androgen therapy presents hepatic oncogenic properties [63, 64]. Screening for HCC in this context is difficult because HCC may occur despite typical radiological patterns of HCA. The α-foetoprotein test is not reliable as this biomarker has been found to be increased in about 85% of FA patients [67]. Close followup by imaging is mandatory for early diagnosis of HCA. When HCA is diagnosed, discontinuation of androgen therapy should be discussed if bone marrow function permits, as tumors may regress if androgens are withdrawn. Regression of HCA has also been reported after BMT. Close followup to depict transformation into HCC is mandatory and should be prolonged as late development of liver tumors (up to 24 years) is possible. Hemorrhagic complications have also been reported even after discontinuation of androgen therapy [49].

Heterozygous germline mutations of the hepatocyte nuclear factor-HNF1 alpha are associated with liver adenomatosis and maturity onset diabetes of the young (MODY 3) [46, 68–71]. Expression of the phenotype is variable for diabetes and adenomatosis. Severe intraperitoneal hemorrhages related to complicated adenomas have been reported, with a fatal issue in a sixteen-year-old girl [69]. Cases of malignant transformation have also been reported [68]. Adenomatosis has been reported in teenage patients with the youngest being 14 years old [48]. Systematic screening should be performed in relatives of patients with liver adenomatosis and should start at the age of ten years. Ultrasonography is a good screening tool, but adenomas may be difficult to diagnose in some patients. MRI with contrast injection should be performed to increase the sensitivity of screening (Figure 4). A CT scan with contrast injection can also be proposed if MRI is not available. Adenomas are often steatotic. Serum α-foetoprotein levels should also be part of the screening. If liver adenomatosis is detected, survey and preventive surgical treatment should be discussed. Criteria that guide treatment include the number and size of the lesions, the presence of symptoms, and the surgical risk incurred by the patient.

Congenital or Surgical portosystemic shunts are associated with HCA, as reported in several cases [22, 23, 72]. Partial or complete diversion of the portal flow through the shunt leads to an abnormal hepatic circulation that may cause hepatocytic proliferation with nodule formation [8]. Regression of adenomas has been observed after closure of CPSS and restoration of portal blood flow (Figure 6).

3.5. Management. It is now well established in adults that complications depend not on the number of lesions but on the histologic subtype and size of the tumor. In adults, complete surgical resection is an effective option for HCA larger than 5 cm or in male or if HCA is associated with GSD. In our personal pediatric experience and in the pediatric literature, HCAs which are frequently larger than 5 cm are resected [38, 40]. Surgical resection should also be discussed in case of β-catenin mutation or uncertain diagnosis. Some authors propose resection of smaller HCA, between 3 and 5 cm, because of reported cases of malignant transformation [53, 73]. The surgical technique will depend on the context, location, and size of the tumor, and it may consist of a tumorectomy, atypical resection, or hepatectomy. Liver transplantation has been proposed by some authors in the case of multiple lesions [48, 74, 75]. In our experience and as suggested by Dokmak and coll. in adult patients, we propose a conservative treatment for multiple HCA or adenomatosis, involving resection of the largest tumors and close followup of the remaining small lesions with iterative resection if necessary. The place of percutaneous ablation using either radiofrequency, cryotherapy, or other techniques is still unclear [76].

Embolization can be performed in the case of hemorrhage.

With predisposing factors such as GSD, androgen steroid therapy, CPSS, or SPSS, regression of HCA has been observed with metabolic control, androgen withdrawn, and closure of the shunt, respectively.

4. Conclusion

Most FNH remain sporadic during childhood, but predisposing factors exist, as follows: long-term cancer survivor (with an increasing incidence) and portal deprivation in CPSS and SPSS. The aspect is atypical on imaging in two-thirds of cases. Biopsy of the tumor and the nontumoral liver is then required. Surgical resection will be discussed in the case of large tumors with or without symptoms. In the case of associated vascular disorder with portal deprivation, restoration of the portal flow will be discussed. This will be done either by closure of a congenital portosystemic shunt or by making a mesenterico-rex bypass in the cavernous transformation of the portal vein in the hope of seeing the involution of FNH.

HCA, which is very rare in children, is frequently associated with predisposing factors such as GSD type I and III, Fanconi anemia especially if androgen therapy is administered, CPSS, and SPSS. Adenomatosis has been reported in germline mutation of HNF1-α. Management will depend on the presence of a predisposing factor and may include metabolic control, androgen therapy withdrawn, or closure of the shunt when appropriate. Surgery is usually performed on large lesions. In the case of adenomatosis or multiple lesions, surgery will be adapted. Close followup is required in all cases.

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Clinical Study

Histological and Immunohistochemical Revision of Hepatocellular Adenomas: A Learning Experience

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Light has been shed on the genotype/phenotype correlation in hepatocellular adenoma (HCA) recognizing *HNF1α*-inactivated HCA (H-HCA), inflammatory HCA (IHCA), and *β*-catenin-activated HCA (b-HCA). We reviewed retrospectively our surgical HCA series to learn how to recognize the different subtypes histopathologically and how to interpret adequately their immunohistochemical staining. From January 1992 to January 2012, 37 patients underwent surgical resection for HCA in our institution. Nine had H-HCA (25%) characterized by steatosis and loss of L-FABP expression; 20 had IHCA (55.5%) showing CRP and/or SAA expression, sinusoidal dilatation, and variable inflammation; and 1 patient had both H-HCA and IHCA. In 5 patients (14%), b-HCA with GS and *β*-catenin nuclear positivity was diagnosed, two already with hepatocellular carcinoma. Two cases (5.5%) remained unclassified. One of the b-HCA showed also the H-HCA histological and immunohistochemical characteristics suggesting a subgroup of *β*-catenin-activated/*HNF1α*-inactivated HCA, another b-HCA exhibited the IHCA histological and immunohistochemical characteristics suggesting a subgroup of *β*-catenin-activated/inflammatory HCA. Interestingly, three patients had underlying vascular abnormalities. Using the recently published criteria enabled us to classify histopathologically our retrospective HCA surgical series with accurate recognition of b-HCA for which we confirm the higher risk of malignant transformation. We also underlined the association between HCA and vascular abnormalities.

1. Introduction

Hepatocellular adenomas (HCA) are rare benign tumors most frequently observed in women on oral contraception [1, 2]. HCA can occur in men on anabolic steroids [3] or be associated with underlying metabolic diseases such as glycogen storage disease [4]. Some associations have also been described with congenital vascular abnormalities of the liver [5–8].

The existence of four different categories of HCA was recently recognized, and the clinical relevance of subtyping these liver lesions according to histological and immunohistochemical features and to molecular alterations was demonstrated [9–15].

HNF1α-inactivated HCA (H-HCA) are associated with *HNF1α* inhibiting mutations leading to the loss of expression of liver fatty acid binding protein (L-FABP) within the lesion as compared with the surrounding liver parenchyma by immunohistochemistry (IHC). These HCA are histologically associated with marked liver steatosis and do not show cytological abnormalities. The second group, the more frequent, is the inflammatory type of HCA (IHCA) associated with the activation of inflammatory pathways, and showing expression of serum amyloid A (SAA) and C-reactive protein (CRP) by IHC. Histologically, IHCA exhibit variable amounts of sinusoidal dilation, inflammation, and ductular reaction. The third subgroup is related to activating mutation of the *β*-catenin pathway (b-HCA) and carries a higher risk of transformation in hepatocellular carcinoma (HCC). The b-HCA can be identified on IHC by a nuclear accumulation of *β*-catenin and by the presence of a strong cytoplasmic staining

for glutamine synthetase (GS). The last group corresponds to the unclassified and less-well understood HCA.

Our aim was to review our series of HCA in light of the new histological and immunohistochemical criteria [11–13, 15] in order to learn how to deal with these lesions prospectively in our current histopathology practice.

2. Materials and Methods

2.1. Patients. A retrospective study of our surgical series of HCA was performed with the help of the Bordeaux group on thirty-one cases retrieved from the archives of our department of pathology. Six additional HCA surgically removed in our institution were then similarly studied, leading to a total number of 37 cases from January 1992 to January 2012.

Clinical data including age, sex, body mass index (BMI), and oral contraceptive (OC) use, number of lesions, and clinical presentation which led to HCA diagnosis were retrieved from the clinical files.

2.2. Histopathological and Immunohistochemical Analyses. Formalin-fixed paraffin-embedded liver specimens were routinely stained with Hematoxylin Eosine (HE) to identify the histopathological characteristics of each one of the HCA. Liver adenomatosis was defined when more than 10 HCA were present within the liver [13, 15, 16]. Then, immunohistochemical staining for cytokeratin 7 (CK7, monoclonal mouse antibody, 1 : 250 dilution, Dako), L-FABP (polyclonal rabbit antibody, 1 : 50 dilution, Abcam), CRP (monoclonal rabbit antibody, 1 : 1500 dilution, Abcam), SAA (monoclonal mouse antibody, 1/30 dilution, Dako), GS (monoclonal mouse antibody, 1/800 dilution, BD Bioscience), and β-catenin (monoclonal mouse antibody, 1/300 dilution, BD Bioscience) were performed according to the recommendations of the Bordeaux group. In cases suspicious of transformation into HCC, immunohistochemical staining of glypican-3 (monoclonal mouse antibody, 1/100 dilution, BioMosaics) was also carried out. HE and IHC staining of 31 cases was analyzed as a training set, with the help of the Bordeaux group. Based on this training, one pathologist (CS) examined subsequently the next 6 cases.

3. Results

The clinical data of the 37 patients are given in Table 1.

The majority of the patients in our series were women (89%). The mean age at diagnosis was 40.9 years with a range from 17 to 68 years of age. In addition, there was one case of HCA surgically removed at 11 years of age in a boy with congenital absence of the portal vein. Twenty of the 33 female patients (66.7%) were on OC. Fifteen patients (44.1%) had a BMI higher than or equal to 25 and none of the patients had diabetes. Fifteen patients (42.9%) presented with symptoms related to intrahepatic hemorrhage of HCA. The other patients were diagnosed either incidentally or during the workup for other nonrelated symptoms. The young boy was followed since several years for portal vein agenesis. HCA was single in 22 patients (59.5%), whereas 3 patients (8.1%) had liver adenomatosis.

Table 1: Clinical data.

Age (years)	11–68
Sex	
Female	33 (89%)
Male	4 (11%)
BMI (kg/m^2)	
<25	19 (55.9 %)
≥25	15 (44.1%)
Unknown	3
OC (33 women)	
OC use	20 (66.7%)
No OC use	10 (33.3%)
Unknown	3
Number of HCA (radiological)	
Single	22 (59.5%)
Multiples	12 (32.4%)
Adenomatosis	3 (8.1%)
Symptoms	
Bleeding	15 (42.9%)
Aspecific/incidental finding	20 (57.1%)
Unknown	2

The histological and immunohistochemical analyses allowed us to classify the HCA of our series of 37 patients into the four recently described categories (Table 2).

Lack of L-FABP expression, suggestive of *HNF1α*-inactivated HCA (H-HCA), was observed in 9 patients (25%). All patients in this group were women, aged from 27 to 68 years; 55.5% had been on OC and 28% had a high BMI. The first manifestation was bleeding in 44.4%. Four women had a single lesion and 1 had liver adenomatosis. The size of the lesions varied from 30 to 100 mm. Followup, available for 7 patients, ranged from 10 to 129 months with all patients alive, one harboring residual HCA.

On HE staining, H-HCA showed a very homogeneous histological aspect. The lesion had a nodular and clear appearance at low magnification. The liver cell plates were regular and composed of large hepatocytes showing moderate-to-marked steatosis and a small dark nucleus. A few compressed liver cell plates with hepatocytes exhibiting an acidophilic cytoplasm were observed in between. There were no atypia, no inflammation, and no sinusoidal congestion. Lipofuscin pigment was abundant in some cases. The adjacent liver was normal in 8 patients and showed moderate steatosis in 1 patient. The absence of L-FABP staining was not always easy to assess. Indeed, in 2 patients, we observed more a lower L-FABP expression by contrast to the adjacent normal liver than a true negative staining. We found it therefore useful to have this internal positive control for comparison. No CRP or SAA staining was observed. Rare scattered hepatocytes expressing CK7 were present mainly around vessels or fibrotic areas. GS staining was negative or sometimes focally expressed at the borders of the adenoma or around vessels, whereas outside the lesion, GS was characteristically expressed in the perivenular areas allowing the recognition of the normal

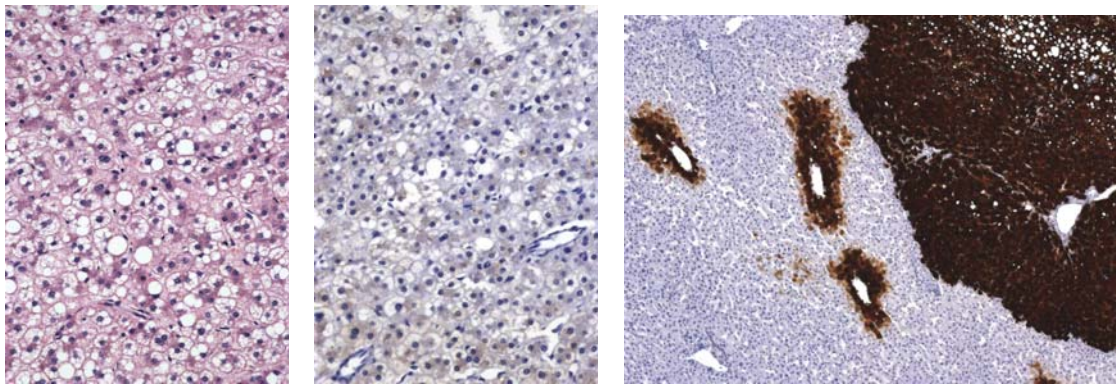
Table 2: Clinical data according to hepatocellular adenoma subtypes. (The patient with both H-HCA and IHCA is not included in the table.)

Characteristics	H-HCA	IHCA	b-HCA	Unclassified
Number of cases	9 (25 %)	20 (55.5%)	5 (14%)	2 (5.5%)
Age (years)	27–68	17–49	11–42	28, 32
BMI (kg/m^2)				
<25	6	8	4	0
≥25	3 (28%)	9 (45%)	1 (20%)	2 (100%)
Unknown	0	3	0	0
Sex				
Females	9	18	3	2
Males	0	2	2	0
Number of HCA (radiological)				
Single	4 (44.4%)	12 (60%)	5 (100%)	1 (50%)
Multiple	5 (1 adenomatosis)	8 (2 adenomatosis)	0	1
Symptoms				
Bleeding	4 (44.4%)	7 (35%)	1 (20%)	2 (100%)
Aspecific/incidental finding	5	11	4	0
Unknown		2		
OC use (32 women)				
Yes	5 (55.5%)	10 (50%)	2 (67%)	2 (100%)
No	3	6	1	
Unknown	1	2		
Size of lesions (mm)	30–100	27–140	50–180	66–100
Followup (range in months)	10–129	10–200	8–120	24–83
Alive without recurrence	6	16	5	2
Alive with recurrence				
Alive with residual HCA	1	2		
Unknown	2	2		

parenchyma. Importantly, one patient had a classical H-HCA showing a strong diffuse GS staining with focal nuclear staining for β-catenin suggesting a subgroup of β-catenin-activated/*HNF1α*-inactivated HCA and was classified within the group of b-HCA (Figure 1). No HCC transformation was observed in this HCA. In one patient, the LFABP-negative HCA contained several granulomas, whereas the adjacent liver was completely normal. The patient was found to suffer from sarcoidosis. One patient had an additional lesion of focal nodular hyperplasia and one patient had multiple HCA in a context of absence of the left portal vein. The nontumoral liver in this patient was characterized by hypoplastic portal veins within the portal tracts. The five patients with multiple lesions showed the same histological and immunohistochemical features in all adenomas.

Inflammatory HCA (IHCA) with expression of both CRP and SAA were found in 20 patients (55.5%). They were 18 women and 2 men, aged from 17 to 49 years, and 50% of the women had been on OC. The BMI was high in 45% of the patients and the first manifestation was bleeding in 35%. Twelve patients had a single lesion and 2 had liver adenomatosis. The size of the lesions varied from 27 to 140 mm. Followup, available in 18 patients, ranged from 10 to 200 months. All patients are alive, two with residual HCA.

In our hands, CRP was more powerful than SAA to stain IHCA. Most of the time, it was strongly and diffusely positive within the adenomas, whereas SAA showed a weaker and more granular staining. However, in some cases it was the reverse and the combination of the two staining was therefore very useful. Histologically, IHCA exhibited variable degree of macrovesicular steatosis and inflammation, whereas sinusoidal dilatation and congestion were common features. At low magnification, they were readily identified by the sinusoidal dilatation, a constant finding, and, when present, pseudoportal tracts containing inflammatory cells and delineated by a prominent ductular reaction underlined by the CK7 staining were also very characteristic. Thick-walled arteries were scattered within the lesions and, at higher magnification, nuclear atypia were often seen. Sixteen cases were very characteristic by histology alone, whereas the diagnosis was only firmly made by IHC in 4 cases. L-FABP staining was always normal and similar to the adjacent nontumoral liver. GS staining was negative or very focally expressed, adjacent to vessels or to the borders of the adenomas, by contrast to its normal perivenular expression outside the lesions. However, importantly, two cases with histological and immunohistochemical features of IHCA showed also a strong and diffuse GS expression. One of them had also a focal nuclear



(a) (b) (c)

Figure 1: β-catenin-activated/*HNF1α*-inactivated HCA ((a), Obj ×20) with negative L-FABP ((b), Obj ×20), and strong positive GS ((c), Obj ×5) staining. Note the normal GS staining around the perivenular areas of the adjacent normal liver on the left.

expression of β-catenin suggesting a subgroup of β-catenin-activated/inflammatory HCA (b-IHCA) and was classified within the group of b-HCA (Figure 2). In the second one, we were not able to identify nuclear β-catenin staining and we did not classify it as a b-IHCA. Two IHCA cases were associated with a lesion of focal nodular hyperplasia. In the patients harboring multiple lesions, all lesions were IHCA. The adjacent liver was completely normal in 16 patients, moderate steatosis was found in 3 patients, and no adjacent liver was available for 1 patient.

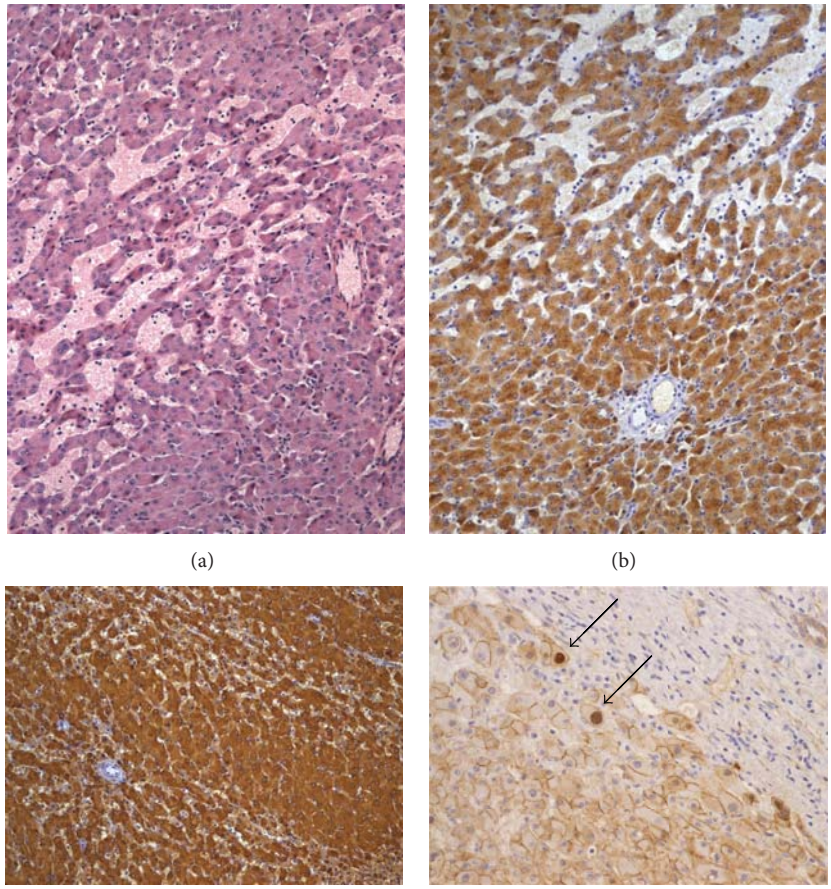
Interestingly, one 47-year-old woman (not included in Table 2) on OC and with a normal BMI was found to have two H-HCA and two IHCA by IHC. Histologically, the four lesions looked very similar and were more suggestive of IHCA, even if two of them lacked L-FABP expression. GS staining was strong in one of the H-HCA and also in one of the IHCA and we found focal β-catenin nuclear staining in both lesions. She is alive without recurrence at 114 months of followup.

Five patients (14%) were identified as having a β-catenin positive HCA (b-HCA). In this group, there were two women on OC and one without, one man who did not take any medication, and one 11-year-old boy. Four patients had a normal BMI and all had a single large lesion (50–180 mm) discovered mostly incidentally in the adults and followed for a few years in the boy because of congenital absence of the portal vein. Imaging techniques revealed absence of the left portal vein in the man. The 5 patients are all alive without recurrence or residual lesion (followup range: 8–120 months).

Histologically, these HCA were variably atypical with hepatocytes of varying sizes exhibiting enlarged and irregular nuclei and with thicker liver cell plates. No significant steatosis was found. In the two male patients, GS staining was strong and diffuse and β-catenin nuclear staining was easy to find. The center of the b-HCA in these two patients showed transformation in HCC with rosette formation, poor differentiation, vascular invasion, and glypican-3 positivity (Figure 3). In addition, in the man, the b-HCA contained important amounts of dark and coarse Dubin-Johnson-like brown pigment that was stained both by PAS and Fontana-Masson. No adjacent liver was available in the man, whereas in the child it showed remodeling of the liver architecture together with absent or hypoplastic portal vein branches within the portal tracts (Figure 3). In one woman, the HCA showed heterogeneous patchy GS staining and the nuclear positivity of β-catenin was difficult to find requiring the use of several slides. Many vessels were present within the lesion, together with very thick liver cell plates and obvious nuclear irregularities. Staining for CK7 was positive in numerous cells and glypican-3 was negative. In these 3 case of b-HCA, there was no expression of inflammatory proteins, except in a few scattered cells and L-FABP staining was normal. As already mentioned, one case of b-HCA showed the histological and immunohistochemical characteristics of an H-HCA (Figure 1) and another b-HCA, those of an IHCA (Figure 2). There were less cytological atypia in these two cases and it is only the diffuse and strong GS staining (Figures 1(c) and 2(c)) that warned us to search for β-catenin nuclear positivity which was very focal (Figure 2(d)).

The last two patients in our series remained unclassified. Their HCA showed a normal L-FABP staining and no staining for SAA, CRP, β-catenin, and GS. They were both found in females, with a mean age of 30 years at presentation. Both were on oral contraceptives and had a high BMI. They presented with hemorrhage complicating their HCA. One of them had a single lesion and one of them had multiple lesions, all the same histologically. Both are alive without recurrence or residual lesion at 24 and 83 months, respectively.

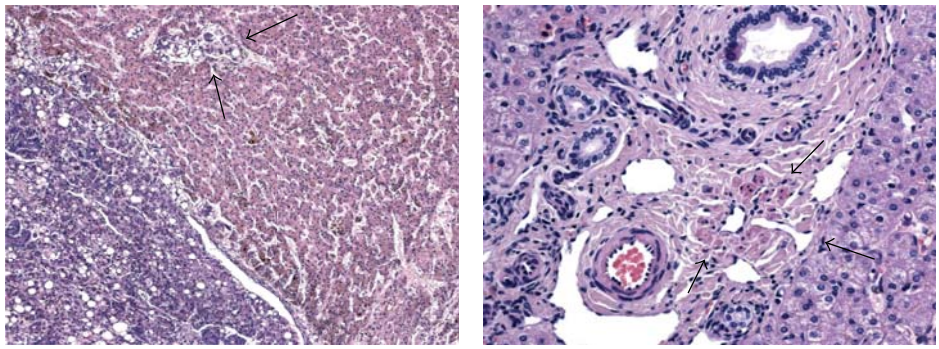
One HCA was composed of small hepatocytes arranged very regularly in thin or slightly enlarged liver cell plates and frequently expressing CK7, whereas the other one showed a highly atypical HCA with large liver cell plates, numerous thick-walled vessels, some steatosis, and worrisome nuclear atypia, with numerous small hepatocytes in between expressing CK7 (Figure 4). This last case was very similar to the one of the woman with b-HCA, except for the absence of GS and β-catenin nuclear staining. The adjacent liver showed mild steatosis in the first patient and was normal in the second one.



(a) (b)

(c) (d)

Figure 2: β-catenin-activated/inflammatory HCA ((a), Obj ×5) with strong SAA ((b), Obj ×5), and GS ((c), Obj ×5) staining showing focal nuclear β-catenin staining indicated by arrows ((d), Obj ×20).



(a) (b)

Figure 3: β-catenin-activated HCA with malignant transformation in a young boy with congenital absence of the portal vein. Microscopic view of the adenoma on the right and of the hepatocellular carcinoma on the left with vascular invasion indicated by arrows ((a), Obj ×10). The adjacent nontumoral liver shows hypoplastic portal vein branches in middle-sized portal tracts (arrows) ((b), Obj ×20).

4. Discussion

Light has been recently shed on the genotype/phenotype correlation in hepatocellular adenoma (HCA) leading to the identification of four different subtypes: *HNF1α*-inactivated HCA (H-HCA), inflammatory HCA (IHCA), β-catenin activated HCA (b-HCA), and unclassified HCA [9–15]. This new classification has been validated [17, 18] and is now included in the 2010 edition of the WHO classification of Tumours of the Digestive System [19]. By reviewing our surgical series of

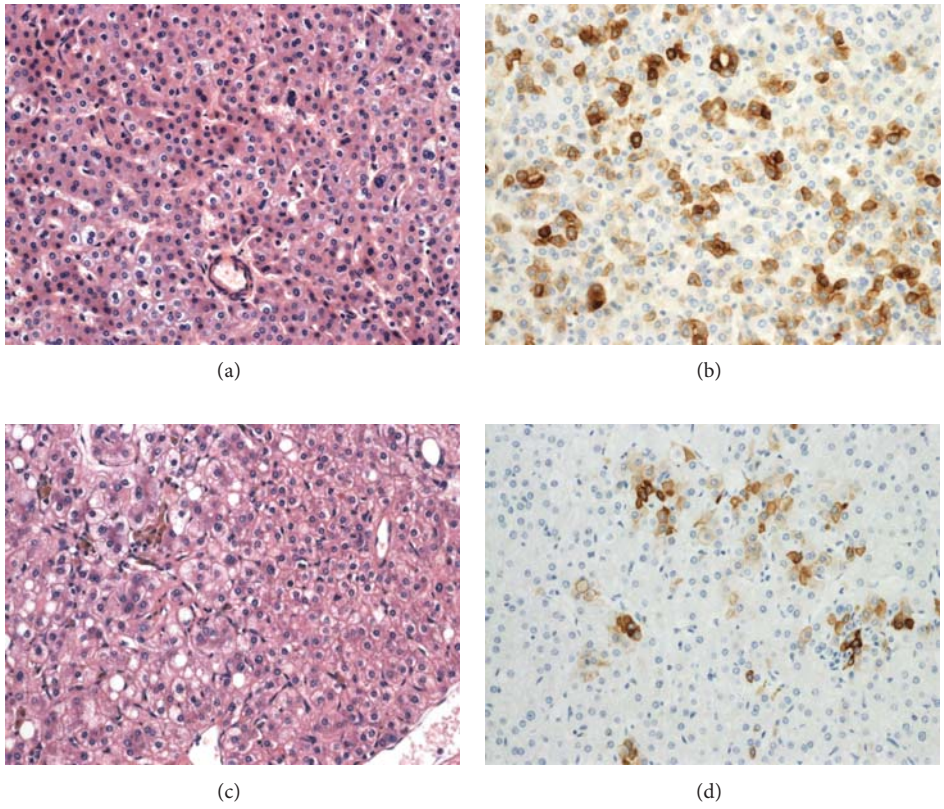


Figure 4: Unclassified HCA (a) with numerous small hepatocytes expressing CK7 (b). Unclassified HCA with worrisome nuclear atypia (c) with numerous small hepatocytes expressing CK7 (d). (Obj ×20).

HCA, we learned how to recognize histopathologically the different subtypes and how to perform and interpret adequately the immunohistochemical staining of these lesions. This learning experience allowed us to classify our retrospective series of 37 patients who underwent HCA surgical resection in our institution.

The clinical characteristics and the frequency of each subtype in our series are similar to what is described in the Western literature [13, 17, 18]. Indeed, we confirmed not only that HCA still occur more frequently in women on oral contraception but also that it can be found in men and in this case be either IHCA or b-HCA. Bleeding is still a frequent complication of HCA that leads to the diagnosis but our study shows that HCA are more and more often found incidentally, probably thanks to the progresses of imaging techniques.

In our series, as in others [13, 17, 18], the larger group of HCA corresponded to the IHCA and this group was more frequently associated with a high BMI. Also in concordance with published data, we saw that multiple lesions were more frequent in the H-HCA subgroup and that b-HCA were large, single adenomas that carry a higher risk of malignant transformation [20], as demonstrated by the presence of HCC in two of our cases. We also confirmed that β-catenin nuclear accumulation can be found in otherwise typical IHCA leading to a subgroup of b-IHCA [15]. Interestingly, we made the same observation in H-HCA, a phenomenon not well known in the literature so far. To understand better this particular finding, molecular studies are required in these cases.

In our experience, most of the HCA were easily classified into one of the subtypes using immunohistochemistry. However, interpretation of the staining requires comparison with nontumoral liver tissue and we would like to strongly recommend having this internal control within the same block. Indeed, L-FABP negative staining in H-HCA can be difficult to assess in the absence of adjacent normal liver tissue. By contrast, CRP can be present in scattered normal hepatocytes outside the lesion. Regarding the identification of IHCA, CRP detection was commonly easier to interpret than SAA because of being strong and diffuse, but for some of the cases it was the reverse and so both stainings are probably useful to identify IHCA when starting to study these lesions. HCA borders are not always easy to identify and GS staining is very helpful to allow the recognition of the normal tissue by labeling the perivenular areas. GS staining is also mandatory to recognize b-HCA in which it is commonly strongly and diffusely expressed. This is extremely helpful knowing that β-catenin nuclear staining can instead be very focal and difficult to find. As exemplified by one of our b-HCA, GS staining can also be patchy within the lesion and this has also to be considered as an abnormal staining suspicious for b-HCA, as shown previously by the Bordeaux group [15, 21]. However, although this type of GS staining is worrisome, its molecular

background is still under investigation. Importantly, a focal GS staining limited to the HCA borders or to perivenular areas within the lesion is not considered as abnormal. In this series, four women showed b-HCA, either isolated or combined with IHCA or H-HCA. Three had a strong diffuse GS staining and one had the patchy GS staining already mentioned. In all but one of these patients, the nuclear labeling for β-catenin was very focal and required the staining of several slides before identification. Therefore, at least in case of a strong diffuse GS staining, β-catenin IHC has to be performed in several slides to identify a b-HCA. Regarding the patchy GS staining, no clear recommendation can be made so far.

Knowing all these possible pitfalls of immunohistochemistry will help the pathologist confronted to the biopsies that will probably be more and more often done in HCA in the next few years before surgical resection. Indeed, identification of the different types of HCA led to new propositions of management of these lesions, including performing a liver biopsy either because the nature of the lesion is uncertain on imaging techniques or to subtype an HCA prior to decide on the appropriate treatment [15, 17, 21–24]. A recent study by the Beaujon group demonstrated the accuracy of classifying HCA by a combination of magnetic resonance imaging and liver biopsy [22] and decision-making models involving liver biopsies are proposed by several authors [21, 23]. To interpret adequately biopsy specimen, it is mandatory to obtain both tumoral and nontumoral tissue for the comparison of IHC staining and to keep in mind that GS staining can be more helpful in identifying b-HCA than nuclear β-catenin positivity that can be very focal [21].

In our series there were some interesting observations of associations between HCA and a particular clinical context. The most interesting one was the curious coincidence observed in 3 patients. A male child and two adults, one female and one male, were found to have an absence of, respectively, the portal vein in the child and the left branch of the portal vein in the two adults. These findings were also associated with a hyperarterialization of the corresponding branch of the hepatic artery. Both males had a single b-HCA transformed in HCC and the female had multiple H-HCA. Focal liver cell lesions including HCA, HCC, focal nodular hyperplasia, and nodular regenerative hyperplasia have been reported in association with congenital absence of the portal vein, also known as congenital extrahepatic portosystemic shunt or Abernethy malformation [5–8, 25–27]. It has been suggested that the decreased venous blood flow combined with an increased arterial blood flow could result in abnormal perfusion of the liver and to uneven vascular perfusion of the liver giving rise to a hyperplastic response, and to a spectrum of nodular lesion including HCA [5, 28]. A study on the complications of congenital portosystemic shunts in children has shown the presence of 13 tumors in 17 patients (76%), 4 corresponding to HCA, one transformed in HCC [26]. Interestingly, by closure of the shunt and restoration of the blood flow, the authors observed a complete or partial regression of the tumors, including regression of one of the HCA. Another recent work examined specifically the liver histopathological lesions that can be found in case of congenital extrahepatic portosystemic shunts in 5 patients [27]. They found two patients with HCC and they described that the nontumoral liver was characterized by absence of portal vein in small portal tracts, absence or hypoplasia of portal vein in medium-sized portal tracts, and remodeling of the liver architecture, all features that we also found in our young patient. In the two adult cases, it is difficult to know whether anomalies of the portal venous system predisposed to HCA or if HCA resulted in some thrombosis and atrophy of the left portal venous system. In the man, no liver parenchyma outside the lesion was available, but in the woman we observed similar anomalies in the portal tracts of the normal parenchyma than those described and observed in case of congenital absence of the portal vein. The concept of anomalous portal tract syndrome [28] has been proposed as a single unifying etiological factor underlying the development of the different types of liver nodules in the context of vascular abnormalities, although for HCA the mechanisms are still poorly understood.

In this series, we also found that one patient had granulomas within her H-HCA leading to a diagnosis of sarcoidosis later on. The presence of granulomas has been described in adenomas [21], but to our knowledge the association with sarcoidosis has not been reported yet. Finally, one patient with b-HCA transformed into HCC showed a pigmented lesion, a very rare phenomenon recently reviewed by the Mount Sinai group who described a middle-aged man with a pigmented b-HCA transformed into HCC with, as in our case, an absence of the left portal vein [29]. This pigment is considered as Dubin-Johnson-like and is stained black with Fontana-Masson whereas iron staining remains negative [30].

In conclusion, using the new histological and immunohistochemical criteria enabled us to classify accurately our retrospective surgical series of HCA with recognition of β-catenin activated HCA. Our series confirms the higher risk of malignant transformation of this particular subtype and underlines the association between HCA and vascular abnormalities. Based on this learning experience that is reproducible in other series of surgically treated HCA, our recommendations to other pathologists would be (1) to always study in parallel the tumoral and nontumoral liver tissue and (2) to perform GS staining on all cases and rely on strong and diffuse GS staining to identify the potential b-HCA that requires further immunohistochemical and/or molecular confirmation. So far, a patchy GS staining is worrisome, but because it is still poorly understood, it requires further molecular investigations before giving any advice.

Conflict of Interests

There is no conflict of interests declared by the authors.

Acknowledgments

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Review Article

Changing Epidemiology of Hepatocellular Adenoma in the United States: Review of the Literature

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Hepatocellular adenoma (HCA) is a benign neoplasm arising from hepatocytes. There is evidence that the inflammatory subtype may be associated with obesity and alcohol use and that men with metabolic syndrome may be at risk for malignant transformation of HCA. We sought to explore the combined experience of US centers as reported in the literature to document the epidemiologic shift in risk factors for HCA formation in the United States, namely, a shift from oral contraceptive pills (OCPs) to an emerging role of obesity as a contributing factor. *Methods*. Publications reporting HCA in the United States were identified through a PubMed search and a review of the literature. We excluded publications prior to 1970, single case reports, and publications for which there was no data available regarding patient characteristics including OCP use and the number of adenomas. *Conclusion*. Whereas earlier reports of HCA in the United States described cases exclusively in women exposed to OCPs, there is a trend towards an increase in HCAs reported in men, HCAs in the absence of OCP use, and increased reporting of multiple HCAs. This may be a result of newer OCP formulations and increasing prevalence of obesity.

1. Introduction

Hepatocellular adenomas (HCAs) are benign hepatic neoplasms that became widely recognized in the 1960s and 1970s following the introduction of oral contraceptive pills (OCP's). Recent advances have identified distinct subtypes based on genotypic classification [1]. These types are (1) hepatocyte nuclear factor-1α (HNF-1α)-mutated HCAs (H-HCA), (2) β-catenin-mutated HCAs (b-HCA), (3) inflammatory HCAs (I-HCA) (which harbor mutations involving the interleukin-6 signal transducer), and (4) unclassified. I-HCAs and H-HCAs account for the majority (80%), while b-HCAs comprise about 10%–15% [2]. Ten percent of I-HCAs also demonstrate β-catenin mutation; however, H-HCAs and b-HCAs are mutually exclusive [3].

HCAs appear as unencapsulated tumors that may be solitary or multiple. Adenomatosis, a term used when greater than 10 adenomas are encountered, can be associated with maturity onset diabetes of the young type 3 (MODY3). Histologically, HCAs are characterized by plates of hepatocytes that lack portal tract elements and are separated by sinusoids. Immunohistochemical staining techniques proposed by the Bordeaux group [1] and validated by others [4–6] aid in the classification of HCAs into the different subtypes which are reviewed briefly in the following.

H-HCAs result from inactivating mutations in the hepatocyte nuclear factor 1 A (HNF1A) gene. Histologic features include marked hepatocellular steatosis, lack of cytologic atypia, and absence of inflammatory infiltrates. This subtype occurs almost exclusively in women and can also be associated with maturity onset diabetes of the young type 3 (MODY3), a condition caused by germline mutations in HNF1A. IHC staining distinguishes this subtype through absent expression of liver fatty acid binding protein (LFABP) in tumoral hepatocytes and normal expression in nontumoral liver.

b-HCAs are characterized histologically by nuclear atypia and pseudoacinar formation. This subtype has been associated with men, androgen treatment, and glycogen storage disease. IHC demonstrates aberrant nuclear beta-catenin staining and strong positive staining for glutamine synthetase. This subtype has been associated with an increased risk of malignant transformation [2]. Twenty to thirty percent of HCAs undergoing malignant transformation show β-catenin mutations [1, 7].

Inflammatory HCAs comprise 40%–50% of adenomas and are the type most commonly associated with OCP use, although obesity and alcohol consumption have also been identified as risk factors [1]. Histologic features include sinusoidal dilatation, inflammatory infiltrates, peliosis, and pseudoportal tracts with thickened arteries and which lack veins and ducts [3]. Prominent ductular reaction may be present. IHC reveals expression of serum amyloid-associated protein A2 (SAA-2) and C-reactive protein. I-HCAs have not been found to be associated with malignant transformation [3].

With the advent of modern imaging, most adenomas that come to attention are discovered incidentally. Patients may also present with abdominal pain or a palpable mass; hemorrhage is a presenting feature in 20%–40%, and malignant transformation is estimated to occur in 4%–10% [17]. For patients with an asymptomatic solitary adenoma, size greater than 5 cm is commonly considered a basis for elective surgical resection to preempt the risk of bleeding or cancer.

1.1. OCPs as a Risk Factor for HCA. OCPs were first introduced in 1960 and initially contained concentrations of estrogen and progestin that were 2–5 times and 5–10 times higher than current formulations [24]. Before long, an association between OCP use and the development of HCA came to light [21, 23]. In 1979, a case-control study from the Armed Forces Institute of Pathology database estimated an annual incidence of 3-4 HCA per 100,000 long-term (>24 months) users of OCPs as opposed to 0.13 per 100,000 in nonusers [20]. Other complications of early generation OCPs (most notably cardiovascular/thromboembolic) were even more problematic, and with refined understanding of the physiology behind OCPs, subsequent formulations with lower hormonal concentrations were rapidly introduced [24] resulting in a markedly decreased incidence of OCP-associated HCAs [25]. Estrogen levels in women on modern-day OCPs are not higher than normal physiologic levels, and the ongoing association of HCAs with OCPs is primarily the result of the ubiquity of OCP use; indeed, it is hard to find women of child-bearing age who have not used OCPs.

1.2. Role of Obesity and Metabolic Syndrome. New risk factors for HCA have emerged in recent years, in particular obesity [9, 26] and metabolic syndrome [5]. Farges et al. demonstrated an increase in incident malignant HCAs in men over a 15-year period whereas the number of HCAs with malignancy in women did not change over time. Six of twelve men with HCA in their series had metabolic syndrome [5]. Bioulac-Sage reported similar findings in their experience-with cases of HCA that presented to a single center over a 20 year period, namely, an increase in overweight/obese men presenting with HCA [26].

I-HCA is associated with obesity and with steatosis in the nontumoral liver. This was first suggested by Paradis et al. who found that most cases of I-HCA (which in the past was called telangiectatic focal nodular hyperplasia) occurred in overweight or obese patients and that steatosis outside of tumors was found in 69% of cases, with moderate/severe steatosis in 30% [27]. The association between steatosis and HCA was confirmed by several other groups [9, 10] including case reports of HCA [28] and adenomatosis [29–31] occurring in the background of nonalcoholic steatohepatitis.

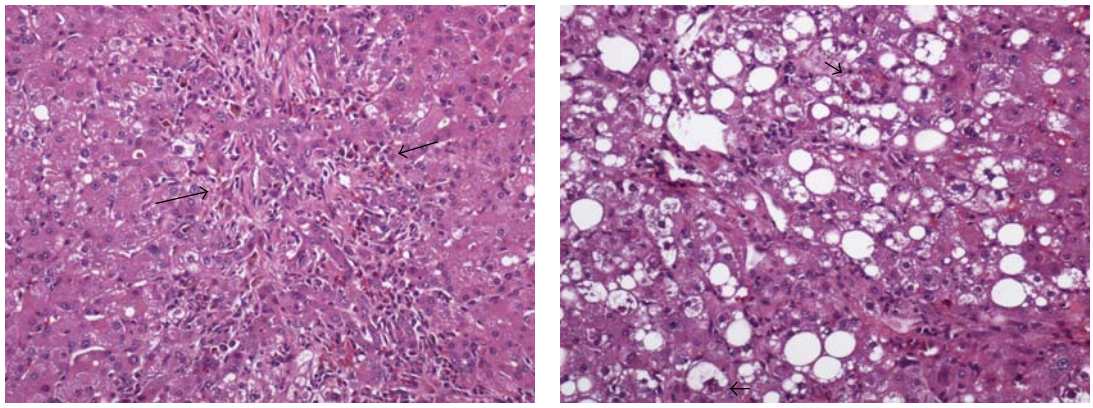
1.3. Adenomatosis and Hepatic Steatosis. Vetalainen reported a review of 94 cases of adenomatosis in the literature and found that 18% had steatosis [31]. Another study [32] found that hepatic steatosis as measured by CT scan was present in 82% of patients with multiple HCAs as compared with 58% of patients with single HCA and 29% in a control group of patients with hemangioma. These series highlight the association between HCAs (in particular multiple HCAs) and steatosis and suggest potential factors that drive both steatosis and formation of HCAs.

We reviewed the combined experience of US centers as reported in the literature to identify whether there is an epidemiologic shift in risk factors for HCA from OCP use to obesity and metabolic syndrome and whether there is a resultant increasing incidence of HCAs in men.

2. Case Series Describing HCA in the United States

Several case series, most of which are single-center experiences, report clinical characteristics of HCA in the United States. Most of the series are single-center experiences from surgical or pathologic databases of resection specimens and are therefore subject to selection bias. Publications reporting HCA in the United States from 1970 onwards were identified through a PubMed search and review of the literature. Published series for which information regarding clinical presentation was available were selected and divided into "early experience" (prior to 1985) and "later experience" (beyond 1985), as summarized in Tables 1 and 2. Whereas earlier case series tended to report single HCAs, nearly all found in women taking OCPs, later series describe more cases presenting with multiple HCAs, more men, and fewer cases associated with OCP use. We explore this in more detail later and highlight recent evidence supporting an emerging role of obesity and metabolic syndrome as a possible factor to explain cases of HCA in the absence of hormonal therapy.

The two largest surgical series of HCA experiences from the United States are a multicenter series [11] and the Pittsburgh experience [12]. Deneve et al. [11] described 124 patients with HCA from 5 different hepatobiliary centers over a nine-year period with an aim to identify risk factors associated with rupture and/or malignant transformation. All of the patients in the series received treatment; the majority



(a) (b)

Figure 1: A 40-year-old man with NASH with severe activity (grade 3 of 3) and transition to cirrhosis (stage 3 of 4). (a) Hepatocellular adenoma containing portal tract-like structures with mild inflammation and marked ductular reaction (arrows). Steatosis is absent (H&E stain, ×100). (b) Nontumoral liver with severe ballooning degeneration of hepatocytes. Some of the ballooned hepatocytes contain Mallory-Denk hyalines (H&E stain, ×100).

(96%) underwent resection while the rest (4%) were treated with embolization. Of those who underwent resection, 25% had evidence of rupture. Hormone use was more common in ruptured versus nonruptured HCA (58% versus 25%, $P = 0.001$). Ruptured HCAs were also larger (10.5 ± 4.5 cm versus 7.2 ± 4.8 cm). Logistic regression analysis identified tumor size >7 cm ($P = 0.001$) and hormone use ($P = 0.007$) as independent risk factors for rupture. No ruptured HCA was <5 cm. Five patients (4%) had HCC; all occurred in HCAs that were >8 cm, and three of five occurred in patients who were taking OCPs. Mean BMI was 27 (±5), and there was no correlation between BMI and risk for rupture. History of hormone use was reported in 55% of patients, which the authors noted was lower than rates of 72%–77% reported in earlier series. Details about patients who developed HCA in the absence of OCP use were not available.

Cho et al. published a single center surgical series from Pittsburgh describing their experience with 41 patients who underwent resection for HCA over a 19-year period [12]. The primary aim was to identify factors which correlate with bleeding and the development of cancer. Most patients (93%) were women, and the median age was 36 years. OCP use was reported in 22 women (54%). Five patients had evidence of steatosis; information on BMI, metabolic syndrome, or other potential risk factors in the individuals diagnosed with HCA in the absence of OCP use was not provided. Rates of hemorrhage and malignancy were 29% and 4.8%, respectively. Both of these series were published before molecular classification of HCA subtypes was available; therefore there is no information provided regarding molecular classification.

2.1. Recent Series Highlighting the Role of Obesity. Recently, Bunchorntavakul et al. described a single-center experience of 60 consecutive patients with HCA over a 5-year period [9]. The primary aim of this study was to investigate whether there is a correlation between obesity and the clinical course of HCAs. Unlike other series which were based entirely on surgical specimens, this series included both patients who underwent resection and patients who were observed in the absence of intervention over a median followup of 2.4 years. Diagnosis in nonsurgical cases was based on imaging and clinical features; pathology was available in 32 patients. Of these, five (16%) had I-HCA. Steatosis and steatohepatitis were found in the nontumoral liver in 28% and 16% of cases, respectively. Eleven (18%) patients were overweight and 33 (55%) were obese. In comparison, the authors note that this is higher than the prevalence of obesity of 27%–34% in an age- and sex-matched population in the same region. Obesity was associated with multiple adenomas (85 versus 48%, $P = 0.007$), bilobar distribution (67% versus 33%, $P = 0.01$), and tumor size progression (33% versus 0%, $P = 0.05$). Unlike other groups, the authors did not note an association between obesity and I-HCA.

The 26 patients who were observed over a median 2.4 years (1–7.3 years) provide a view, albeit limited, of the natural history of HCA. Resection was advised for HCAs >5 cm, bleeding, or inability to exclude malignancy; asymptomatic HCAs <5 cm were imaged every 6–12 months. Patients taking OCPs were advised to stop. HCAs remained stable in 77% and regressed in 4%; progression was observed in 19%, with progression more likely in obese patients (33.3% versus 0, $P = 0.05$). In summary, this study demonstrates an association between obesity and multiple adenomas as well as progression of adenomas.

Two case reports describe hepatic adenomatosis in the setting of steatohepatitis [29, 30] in the USA. Brunt described a 54-year-old woman with elevated BMI and prior OCP exposure who presented with abdominal pain and adenomatosis. The second case, described by our center, highlights a 53-year-old woman with BMI 24 and OCP use who presented with adenomatosis and background of moderate steatohepatitis. IHC showed positive SAA staining consistent with I-HCA. Figure 1 illustrates a case from our center of a 40 year old

Table 1: Published series of hepatocellular adenoma in the United States (1985–2011).

Author Year of publication Study duration	*n*	Sample population	Hormonal therapy (%)	Women (%)	Mean age	Single	Multiple	Presenting symptoms	Hemorrhage	Malignancy	Treatment
Deodhar et al. [8] 2011 (2006-2007)	8	Single-center series, bland embolization	4/8 (50%)	7/8 (87.5%)	39	1 (12.5%)	7 (87.5%)	6 (75%) asymptomatic 1 (12.5%) rupture 1 (12.5%) abdominal pain	1/8 (12.5%)	0	Bland embolization (all)
Bunchorntavakul et al. [9] 2011 (2005–2010)	60	Single-center series	45 (75%)	58 (97%)	36 (median)	28%	72%	36 (60%) incidental 15 (25%) abdominal pain 7 (11.7%) bleeding 2 (3.3%) abnormal LFTs	7 (11.6%)	0	17 resection 9 TAE 8 sequential TAE + resection 26 observation
Mounajjed and Wu [10] 2011 (1994–2010)	35	Single-center series from pathology database	23/29 (79%)	30 (86%)	39	15 (43%)	20 (57%)	NR	10/49 (20%)	0	Resection (all)
Deneve et al. [11] 2009 (1997–2006)	124	Multicenter surgical series	55%	116 (94%)	39	77 (62%)	47 (38%)	NR	31 (25%)	5 (4%)	119 resection 5 embolization 8 RFA (during resection)
Cho et al. [12] 2008 (1988–2007)	41	Single-center surgical series	22 (54%)	38 (93%)	36 (median)	NR	NR	29 (70%) abdominal pain 7 (17%) incidental (on imaging) 3 (7%) abnormal LFTs 2 (5%) incidental (laparoscopy)	12 (29%)	2 (4.8%)	Resection (all)
Charny et al. [13] 2001 (1992–1999)	12	Surgical series	NR	10 (83%)	34	NR	NR	8 (67%) "symptoms" 3 (25%) abnormal LFTs	NR	1/8	8 resection
Reddy [14] 2001 (1983–1997)	25	Single-center series, database of cases from radiologic or surgical diagnosis	21/22 (95%)[1]	25 (100%)	33	16 (64%)	9 (36%)	3 (12%) rupture	3 (12%)	1 (4%)	19 resection 1 unresectable 2 no intervention
Ault et al. [15] 1996 (1985–1995)	11	Single-center surgical series	9/11 (82%)	10 (91%)	37.6	8 (73%)	3 (27%)	10 (91%) abdominal pain	4 (36%)	3 (27.2%)	4 resection 4 observation 4 embolization
Nagorney [16] 1995 (1989–1993)	24	Single-center series	9/22 (41%)	22 (92%)	36	20 (83%)	4 (17%)	19 (79%) symptomatic	4	2[2]	19 resection 4 observation 1 liver transplantation

TAE: transarterial embolization, NR: not reported.
[1]No data on OCP use in 3.
[2]One HCC, one intrahepatic cholangiocarcinoma.

Table 2: Published series of hepatocellular adenoma in the United States (1973–1985).

Author Year of publication Study duration	*n*	Sample population	Hormonal therapy (%)	Women (%)	Mean age	Single	Multiple	Presenting symptoms	Hemorrhage	Malignancy	Treatment
Mays and Christopherson [18] 1984 (1973–1984)	71	Single-center surgical registry	83%	100%	30	NR	NR	(36.6%) mass (31%) hemoperitoneum (24%) pain (5.6%) incidental (2.8%) unknown	NR	NR	
Weil et al. [19] 1979 (1970–1978)	8	Single-center surgical series	4 (50%)	7 (88%)	24	8 (100%)	0	6 fever 6 abdominal pain 3 palpable abdominal mass	4 (50%)	0	Resection
Bourne Rooks et al. [20] 1979 (1957–1976)	79	Database of pathology referrals	73 (92%)	79 (100%)	30.4	NR	NR	34% intraperitoneal bleeding 15% intratumoral hemorrhage 47% abdominal mass 19% abdominal pain	49%	0	Resection
Edmondson et al. [21] 1976 (1955–1976)	42	Single-center surgical series	31/36[1] (86%)	42 (100%)	NR	NR	NR	12 (28%) pain 18 (43%) palpable mass 2 (4.7%) incidental	10 (24%)	0	41 resection
Ameriks et al. [22] 1975 (1969–1973)	8	Single-center surgical series	8 (100%)	8 (100%)	33.6	7	1	3 hemoperitoneum 3 abdominal pain, intrahepatic hemorrhage 3 palpable mass	6 (75%)	0	Resection
Baum et al. [23] 1973	7	Case series	7 (100%)	7 (100%)	28.7	7	0	5 abdominal pain 2 right upper quadrant mass	5	0	Resection

NR: not reported.
[1]Data on OCP use not available in 6 cases.

man with HCA arising in the background of nonalcoholic steatohepatitis (NASH). These along with the series from Bunchorntavakul hint at a rising incidence of HCA in the setting of obesity and/or steatohepatitis.

3. Comparison of the US Experience in relation to the World

There are single-center series from France [5, 26] demonstrating a rising incidence of HCA in men. There are no single-center US series showing this, but comparison of published series from 1970 to 1985 shown in Table 2 with the series from 1985 to 2011 shown in Table 1 reveals a trend similar to what has been described in Europe. HCAs in men were rarely reported in the early series published from 1970 to 1985 (Table 2) whereas the later series published from 1985 to 2011 describe more male patients and more malignancies. This discrepancy cannot be explained entirely by the introduction of molecular and immunohistochemical studies which were introduced after 2006. One hypothesis is that increasing prevalence of obesity may play a role in this epidemiologic shift towards incident cases of HCA in men. Interestingly, studies from both China [33] and Japan [6] report a preponderance of HCA in men, which may reflect different practices with OCP use. The studies included patients with Hepatitis B; therefore some cases which were classified as HCA may in fact have been well-differentiated HCC.

The reader is directed to the study by Balabaud et al. in this volume which describes molecular classification of HCAs from different centers around the world. The Mount Sinai experience of 61 resected HCAs cases over a 12-year period (1990–2012) shows a preponderance of I-HCA (44%) followed by H-HCA (33%) and unclassified HCA (16%) and only one case (2%) of b-HCA (unpublished data). This is a lower proportion of b-HCA cases than what has been described in Europe (10%–15%). One case that had features of both I-HCA and b-HCA had a focus of hepatocellular carcinoma.

4. Conclusions

There appears to be a rising incidence of HCA diagnosis as reflected in the trend towards publication of larger case series over recent years, though this is due in large part to increasing discovery of incidental HCAs with widespread use of imaging. Alternatively, it could be that with time, centers have accrued a larger population to report.

The impact of modern OCPs on the development of HCA is probably marginal; on the other hand, in concordance with reports from European groups, there is increasing evidence to suggest obesity and metabolic syndrome as emerging risk factors for HCA. Multiple HCAs/adenomatosis in association with these risk factors appear to be increasing as suggested by case reports in the literature. Furthermore, European groups [5, 26] have highlighted recent cases of malignant transformation of HCA in men with metabolic syndrome.

The prevalence of obesity in the USA has risen dramatically from around 14% in the 1970s to over 35% now [34, 35]. Nonalcoholic fatty liver disease (NAFLD) is now recognized as the hepatic manifestation of metabolic syndrome and is emerging as a common cause of chronic liver disease in parallel with rising obesity trends. It is unclear whether the shift in epidemiology of HCAs and recent reports of HCA in the setting of steatohepatitis are related directly to obesity and NAFLD or whether this merely reflects coincident epidemiologic changes in the population at large. One potential mechanism linking obesity with HCA formation includes increased levels of adipokines such as IL-6. This could lead to formation of inflammatory HCAs which have been found to demonstrate increased activation of the IL-6 signalling pathway [36]. Further studies demonstrating an association between I-HCA and obesity would support this hypothesis. Other mechanisms which could link obesity with HCA formation include the hyperestrogen state that is associated with obesity.

Additional single-center or multicenter longitudinal studies that include both surgical and nonsurgical cases are warranted to examine the evolution of risk factors for HCA in the United States. Future studies should include BMI and metabolic syndrome in descriptions of cases, focusing on both men and women, and should include data on IHC staining to facilitate molecular classification.

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Research Article

Value and Limits of Routine Histology Alone or Combined with Glutamine Synthetase Immunostaining in the Diagnosis of Hepatocellular Adenoma Subtypes on Surgical Specimens

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Immunohistochemistry is a valid method to classify hepatocellular adenoma (HCA). The aim was to test the performance of routine histology combined to glutamine synthetase (GS) staining to identify the 2 major HCA subtypes: HNF1α inactivated (H-HCA) and inflammatory HCA (IHCA). 114 surgical cases, previously classified by immunohistochemistry, were analysed. Group A comprised 45 H-HCAs, 44 IHCAs, and 9 β-catenin-activated IHCAs (b-IHCA), and group B, 16 b-HCA and unclassified HCA (UHCA). Steatosis was the hallmark of H-HCA. IHCA and b-IHCA were mainly characterized by inflammation, thick arteries, and sinusoidal dilatation; b-IHCA could not be differentiated from IHCA by routine histology. Group B was identified by default. A control set (91 cases) was analyzed using routine and GS stainings (without knowing immunohistochemical results). Among the 45 H-HCAs and 27 IHCAs, 40 and 24 were correctly classified, respectively. Among the 10 b-IHCAs, 4 were identified as such using additional GS. Eight of the 9 HCAs that were neither H-HCA nor IHCA were correctly classified. *Conclusion*. Routine histology allows to diagnose >85% of the 2 major HCA subtypes. GS is essential to identify b-HCA. This study demonstrates that a "palliative" diagnostic approach can be proposed, when the panel of specific antibodies is not available.

1. Introduction

Hepatocellular adenomas (HCAs) are rare benign tumors. Molecular data have brought new insight in the characterization of this disease. They allow the distinction of focal nodular hyperplasia (FNH) from HCA and the identification of 2 major HCA subtypes which represent more than 80% of all HCAs, namely, HNF1A-mutated HCA (H-HCA, 35–40%) [1], and inflammatory HCA (IHCA, 50–55%) [2]; 10% of IHCAs being also β-catenin mutated (b-IHCA). The remaining HCAs are the β-catenin-mutated HCA (b-HCA, 10%) [3] and the unclassified HCA (UHCA, which account for less than 10%).

The immunohistochemical (IHC) classification of HCA subtypes was derived from the above-mentioned molecular characterization and showed a good correlation with the molecular data [2]. Using specific IHC markers, such as liver fatty acid-binding protein (LFAPB), C reactive protein (CRP) or serum amyloid A (SAA), glutamine synthetase (GS), and β-catenin, it is possible to identify all HCA subgroups with good confidence. Among these markers, GS is of major importance to identify patients at high risk of malignant transformation. Indeed, abnormal GS staining [4] is a strong argument to suggest β-catenin activation. Unfortunately, the rarity of these tumors in routine practice leads to the low availability of the specific IHC markers. To overcome this problem, the possibility to identify the 2 major HCA subtypes using standard histological techniques has not been tested.

The aim of this study was (1) to test routine histology in the diagnosis of HCA subtypes and (2) to appreciate

the contribution of GS combined with routine histology in the diagnosis of HCA subtypes.

2. Materials and Methods

2.1. Patients and Tissue Samples. All surgically removed HCA cases were retrieved from our files from January 2000 to November 2011. We excluded cases with specific etiologies such as glycogenosis, male hormone administration (since they are very particular and rare conditions), cases of obvious hepatocellular carcinoma (HCC) possibly related to HCA (but without formal confirmation for a previous HCA) and cases with massive hemorrhage or necrosis (but without sufficient nonnecrotic tissue available).

For all patients (114 cases), the following data were available:

(1) clinical data: sex, age, body mass index (BMI), oral contraception (OC), and imaging data (number and size of nodules), as well as liver enzymes (AP, GGT) and CRP in blood,

(2) routine stainings on paraffin sections in tissue areas devoid or with minimal necrosis or hemorrhage,

(3) IHC stainings. Since 2007, IHC has been performed prospectively; prior this date, it had been performed retrospectively in all cases, according to previous published studies [2]; the HCA classification was based on IHC.

Briefly, taking into account our own experience previously published in pathology textbooks showing the very good correlation between molecular analysis and IHC for H-HCA and IHCA [5, 6]: LFABP negativity in tumor (T) and positivity in nontumoral liver (NTL) was interpreted as H-HCA; SAA or CRP staining positive in T and negative in NTL was interpreted as IHCA (even though SAA/CRP detection could be more or less intense and homogeneous). Once the diagnosis of H-HCA was made, SAA or CRP staining was not mandatory, and once the diagnosis of IHCA was made, LFABP staining was not mandatory in such cases. The expression of GS, a target gene of β-catenin, was studied in all cases. GS staining was quoted as negative in the absence of abnormal expression; and in this situation, β-catenin staining was not mandatory. GS staining in T always differed from the normal distribution of GS which is limited to a few rows of centrilobular hepatocytes in the NTL. In T, GS could be either totally absent or restricted to the border between T and NTL and/or around some persistent veins within T. Abnormal expression of GS characterized in the whole nodule by a strong positive staining diffuse or patchy is easy to interpret but is more difficult to interpret when the staining is faint or focal, limited to individual or groups of positive hepatocytes irregularly distributed within the tumor. In any case, abnormal GS staining was considered as a strong argument to suggest β-catenin activation [3, 4]. In these cases, aberrant β-catenin nuclear labelling allowed the final diagnosis of b-HCA or b-IHCA. However, the absence of labelled nuclei on the section did not rule out this diagnosis because the number of labelled nuclei can be heterogeneously distributed, extremely limited, or even absent on the section. When all the specific IHC markers were negative, the HCA was termed UHCA.

Another cohort of 91 HCAs was analyzed for external validation. It corresponded to cases not included in the above series and concerning cases from our center (prior 2000) or from cases sent for advice. For each case, 2 slides of H&E and GS (including the tumoral and nontumoral part) were available.

2.2. Methods

2.2.1. Identification of Major Pathological Features Using Routine Histological Criteria in 114 HCA Cases Previously Classified by IHC. Cases were divided into 2 groups. Group A included H-HCA cases and IHCA (β-catenin and non β-catenin activated) cases. Group B included all other cases. For each group and subgroup, we analysed the clinical, imaging, and biological data. Routine stainings (H&E, trichrome) as well as CD34, keratin (K)7 immunostainings of the 2 groups were reviewed (T and NTL) according to a flowchart (see Table 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2013/417323) filled out by an experienced liver pathologist (PBS). Different pathological items were checked such as steatosis, sinusoidal dilatation, congestion, inflammation, thick arteries, ductular reaction. Steatosis and sinusoidal dilatation were graded semiquantitatively as indicated in supplemental Table 1. In the NTL, the liver was considered as normal or steatotic (with or without NASH).

2.2.2. External Validation: Identification of HCA Subtypes from the Cohort (91 Cases) Using Standard and GS Staining. Routine slides from HCA previously classified into subgroups using IHC were analyzed blindly by 2 observers (PBS and CB), without knowing IHC results obtained previously. For each case the following diagnosis based on standard features was: H-HCA, IHCA, and b-IHCA with 3 possibilities: yes, no, and possibly for each. For each case the pathological items had to be filled (supplemental Table 1). If the features were obvious, the diagnosis was certain: H-HCA, IHCA (group A) or another type (group B). The diagnosis of H-HCA was uncertain when the result of the reading was H-HCA "possibly," IHCA "no;" the same was true for IHCA. IHCA cases could be also β-catenin activated if GS staining was abnormal. Abnormal GS staining in HCA without characteristics of H-HCA or IHCA was a strong argument to suggest b-HCA. In exceptional cases, we observed abnormal GS staining in H-HCA. Because of the rather straight criteria defined to categorize cases, disagreement among observers was extremely rare, and when it happens, slides were reviewed and a consensus reached.

2.2.3. Statistics. Disparities between groups according to age, BMI score, size, and the number of nodules were analyzed with unpaired t-test. Abnormal liver enzyme and CRP levels in each group were compared using ANOVA test. Fisher's exact test was used to compare different types of pathological

Table 1: Pathological data of HCA subtypes.

	H-HCA ($n = 45$)	IHCA ($n = 44$)	b-IHCA ($n = 9$)	b-HCA ($n = 4$)	UHCA ($n = 12$)
Steatosis	43	17	1	1	2
>60%/30–60%/10–30%	11/19/13	1/8/8	0/0/1	1/0/0	1/1/0
Focal/spread/diffuse	9/22/12	15/2/0	1/0/0	1/0/0	2/0/0
Sinusoidal dilatation	11	40	9	0	3
Major/moderate/mild	0/4/7	12/19/9	3/3/3	0	0/1/2
Focal/spread/diffuse	10/1/0	23/12/5	8/1/0	0	2/1/0
Peliosis	9	10	5	0	3
Pseudo-PT	1	40	6	0	0
Inflammation	0	43	7	1	0
Ductular reaction	0	31	4	0	2
Remodeling	5	7	3	1	3
Cytological abnormalities	2	0	0	0	2
Micro-HCA*	24	16	1	0	0
Others types of nodules	10	4	1	1	0
NTL					
Steatosis	2	12	2	0	3
Sinusoidal dilatation	1	6	1	1	2
NASH	0	1	0	0	0
Fibrosis	0	1	2	0	1
Portal embolisation	0	4	1	1	0

HCA: hepatocellular adenoma; H-HCA: HNF1α mutated HCA; IHCA: inflammatory HCA; b-IHCA: β-catenin mutated IHCA; b-HCA: β-catenin mutated HCA; UHCA: unclassified HCA; PT: Portal Tract; *: at distance of main tumor; NASH: non alcoholic steatohepatitis; NTL: non tumoral liver.

abnormalities (steatosis and sinusoidal dilatation). P values lower than 0.05 were considered as significant. Kappa statistic was used to measure the level of agreement between the standard histological including GS versus the complete set of immunohistochemical techniques to estimate proportions of HCA subtypes.

3. Results

3.1. Major Pathological Criteria of the HCA Subtypes Using Routine Histological Criteria (114 Cases). Amongst the 114 cases analysed by standard morphology and IHC, there were 98 cases in group A: 45 H-HCAs, 53 IHCAs (9 were b-IHCAs) and 16 cases in group B: 4 b-HCAs and 12 UHCAs. The relevant clinical, biological, and pathological data are shown in Figures 1 and 2, Table 1 and supplemental Table 2.

3.1.1. Group A

H-HCA. This subgroup included 45 patients, all women; 84.4% were under OC; the median age was 40. The median size of the largest nodule was 5.6 cm, and nodules were multiple in 62.2%. 75% of the patients had normal BMI; CRP and AP were rarely elevated, and 22.2% had mild elevation of GGT. Interestingly enough, in 8 patients, micro/small H-HCA (<1 cm) were incidentally discovered on the surgical specimen removed for large tumor(s) of different types including focal nodular hyperplasia (4 cases), 1 angiomyolipoma, 1 HCC; in other 2 cases, there was a past history of melanoma, and the small nodules were suspected to be metastases.

Steatosis was the hallmark of H-HCA, which usually exhibited a very characteristic aspect with lobulated contours (Figures 2(a) and 3(a)). Steatosis was present in 95.6% of cases: severe (>60%), moderate (30–60%), or mild (10–30%) in 25.5, 44.2, and 30.2% respectively; it was totally absent in 2 cases only (Figure 3(b)). Of note, steatosis was spread and/or diffused in 79%. Most of the time, steatotic hepatocytes were intermingled with clear hepatocytes which were predominant in 2 cases, with mild/focal steatosis. Sinusoidal dilatation was present in 24.4% and mild in the great majority of cases. In two cases a few pseudoglandular arrangements were noticed without cytological abnormalities. Micro-H-HCA nodules were observed in surrounding liver in 53.3% of the cases. Other types of nodules (FNH and hemangioma) were associated in 22.2% of cases. NTL was globally normal (Figure 2(d)).

IHCA. This group included 44 patients (39 women and 5 men) (Figures 1(a)–1(e)). The median age was 41.5; 92.3 of women were under OC. The median size of the largest nodule was 6 cm, and nodules were solitary in 63.6%. 54.5% of the patients have raised BMI (29.5% > 30). BMI scoring revealed a strong association between IHCA and high BMI score (Figure 1(a), $P < 0.05$). CRP and GGT were raised in 68.2 and 75%, respectively; AP, GGT, and CRP were higher than in the other HCA groups (H-HCA/b-HCA/UHCA ($P < 0.01$). The hallmark of IHCA was the inflammation (presence of

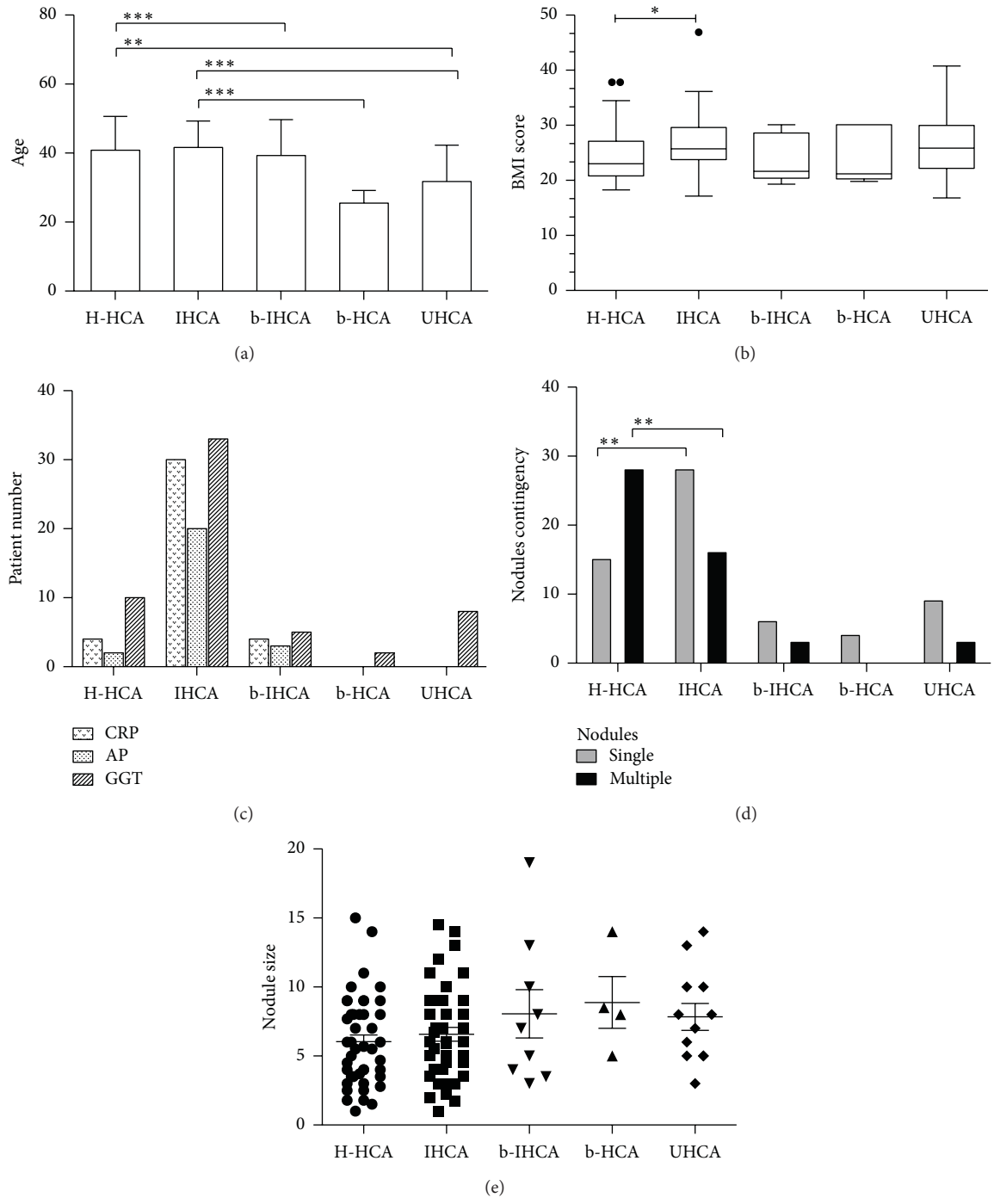


Figure 1: Graphic representation of age (a), BMI score (b), liver enzymes and CRP (c), nodules contingency (d), and nodules size (e) in different HCA subtypes. One asterisk: P value < 0.05; two asterisks: P value < 0.01; three asterisks: P value < 0.001.

inflammatory cells mainly around pseudoportal tracts, sometimes in foci dispersed inside the tumor) and pseudoportal tracts (with thick-walled arteries) present in 97.7 and 90.9%, respectively, (Figure 2(b)), as well as sinusoidal dilatation, which was major or moderate in 30 and 47.5% respectively but focal in 57.5% (Figures 2(c) and 4(a)). Steatosis was present in 38.6% of cases but focal in 88.2% and moderate in 47% (Figures 2(a) and 4(b)). Micro-IHCA were found in 36.4% in surrounding liver of the resected specimen. Surprisingly, in 3 cases, micro H-HCA (<5 mm, solitary or multiple in 2 or 1 cases, resp.) confirmed by lack of LFABP expression was incidentally observed on the surgical specimen at distance of IHCA. Other types of nodules (FNH and hemangioma) were present in 9.1%. The non tumoral liver was steatotic in 27%, and mild sinusoidal dilatation was observed in 13.6%; both steatosis and sinusoidal dilatation were more

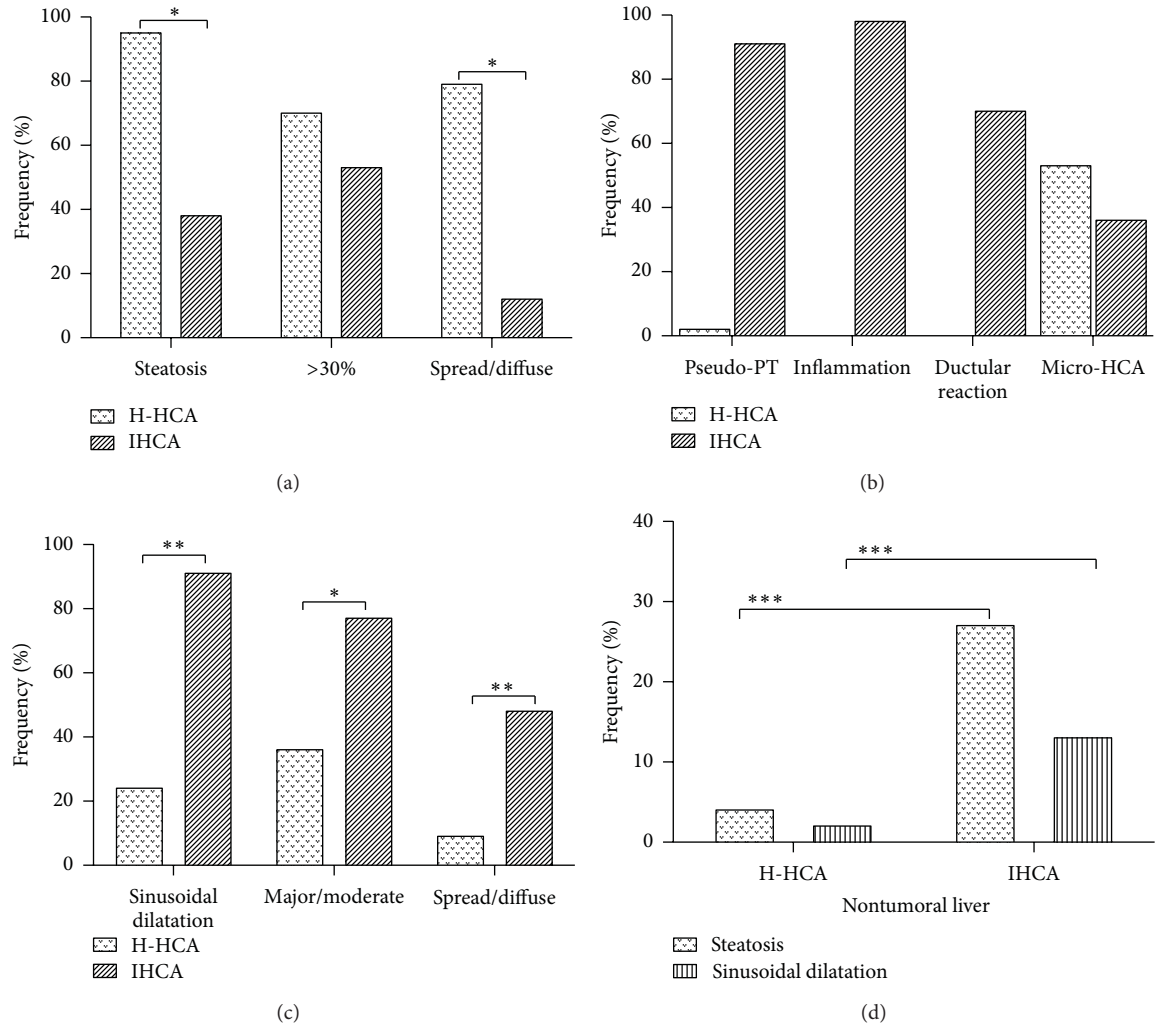


Figure 2: Frequency (%) of various liver pathological abnormalities in the tumoral liver (a–c) and steatosis, sinusoidal dilatation in the non tumoral liver (d) in patients with H-HCA and IHCA. One asterisk: P value < 0.05; two asterisks: P value < 0.01, three asterisks: P value < 0.001.

frequently observed in IHCA group in comparison to others (Figure 2(c), $P < 0.001$).

Comparison between the H-HCA and IHCA groups showed that multiple nodules were more frequently observed in the H-HCA group than in the IHCA group ($P < 0.05$, Figure 2(d)). It is important to note that inflammatory cells, pseudo-PT, or ductular reaction were rare, often not detected in other HCA subgroups than IHCA (Figure 2(a)). However, due to the low number of patients in b-IHCA group, no significance difference was observed when compared to H-HCA group.

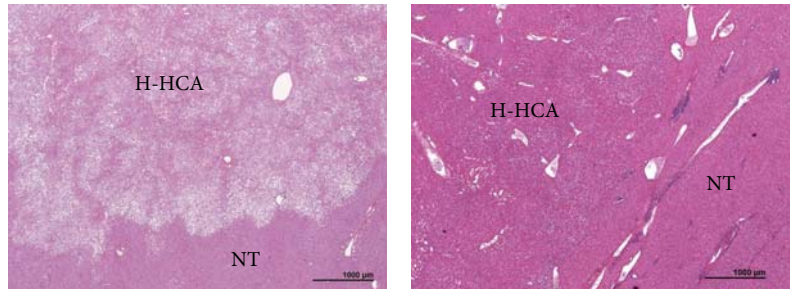
In summary, considering standard histological features, steatosis (frequency and area) and sinusoidal dilatation (frequency and area) may represent the hallmark of H-HCA and IHCA, respectively, (Figure 2, $P < 0.05$ and $P < 0.01$, resp.).

b-IHCA. This group included 9 patients (3 men) and represented 17% of all IHCAs. Clinical abnormalities similar to that observed in IHCA were found (Figure 1). The standard pathological data were identical to IHCA (Table 1, Figure 4(c)), and therefore b-IHCA could not be identified as such using standard stainings; however, abnormal GS staining helps for the right diagnosis (Figure 4(d)).

3.1.2. Group B

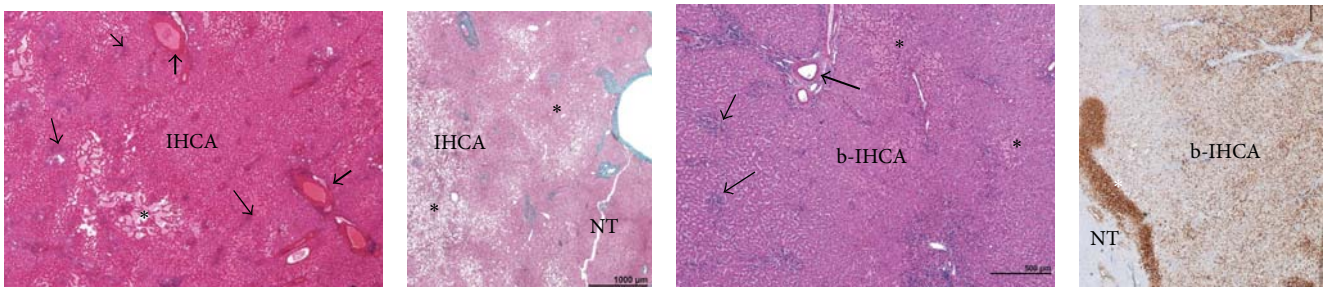
Other Subtypes. It included 16 patients, all women (4 b-HCAs and 12 UHCAs, median age 26 and 26.5, resp.). b-HCA subtype was more frequently detected in young patients (age < 25; $P < 0.01$, Figure 1(d)) than in other HCAs subtypes. Neither specific pathological features nor cytological abnormalities were observed. Areas with many thin dilated veins were observed in 3 cases. In all these 4 b-HCA cases, GS staining was patchy, as defined above (Figure 4(d)).

In UHCA, the median age was 32.9. In half cases, UHCA occurred in young women (mean age 24.5). 50% were overweight/obese. UHCAs were detected often during complications (hemorrhage and necrosis) associated with



(a) (b)

Figure 3: HNF1α inactivated HCA (H-HCA)—(a) H&E: typical aspect with diffuse steatosis, thin vessels, and lobulated contours, contrasting with the absence of fat in the nontumoral liver (NT); (b) this nodule of H-HCA does not contain fat, and sinusoids are slightly dilated.



(a) (b) (c) (d)

Figure 4: (a-b): Inflammatory (IHCA)—(a) typical aspect of IHCA with dispersed inflammatory foci (thin arrow), thick arteries (thick arrow), and areas of sinusoidal dilatation/peliosis (asterisk); (b) this nodule contains irregular areas of steatosis (asterisk); the limits of the tumor (IHCA) from the non tumoral liver (NT) are not visible on standard staining. (c-d) *β-catenin activated inflammatory HCA (b-IHCA)*—(c) typical aspect of IHCA on H&E with inflammatory foci (thin arrow), thick arteries (thick arrow), and areas of sinusoidal dilatation (asterisk); (d) patchy positivity of glutamine synthetase immunostaining in tumor (b-IHCA), contrasting with nontumoral liver (NT).

large tumors. No specific pathological features usually existed (Figure 5(a)), but focal steatosis and sinusoidal dilatation were observed in 16.7 and 25%, respectively (Table 2). In 2 cases, numerous thin dilated veins were focally observed. In 2 cases, a few pseudo glandular arrangements were noticed. In 6 cases, tumoral nodules diffusely expressed CD34 (Figure 5(b)). In all cases, GS staining was normal.

3.2. External Validation: Identification of HCA Subtypes from the Cohort (91 Cases)

3.2.1. Group A. Among the 45 H-HCA, 40 were classified correctly (H-HCA positive and IHCA negative). There were 3 false negative readings (H-HCA negative and IHCA +/or possible/or negative. There were 2 cases with no formal diagnosis (H-HCA possible and IHCA possible). In 3 cases correctly classified H-HCA, GS was abnormal, with mild and patchy staining irregularly distributed within the tumor.

Among the 27 IHCAs, 24 were correctly classified, as well as 4 out of the 10 b-IHCAs. Interestingly enough, none of the b-IHCAs were identified without GS. There were 3 false negative readings: 1 in the IHCA and 2 in the b-IHCA. There were 6 cases with no formal diagnosis (1 in IHCA and 4 in the b-IHCA series. Among the 27 IHCAs, GS was abnormal in 1 case.

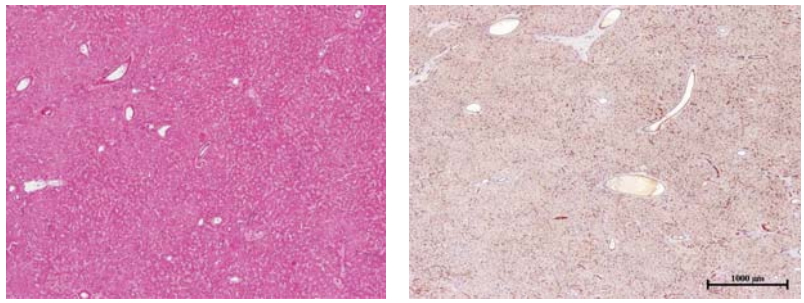
Table 2: Phenotypic classification of hepatocellular adenoma: routine histology and immunohistochemistry (IHC) and molecular biology.

Routine histology	In favor of	IHC
(i) Diffuse steatosis (ii) Lobulated contour (surgical specimen) (iii) No criteria for IHCA	H-HCA	Lack of LFABP $\rightarrow$ H-HCA
(iv) Inflammation (v) Sinusoidal dilatation (vi) Pseudoportal tracts (with thick arteries) (vii) Ductular reaction	IHCA*	CRP/or SAA + $\rightarrow$ IHCA CRP/or SAA +/GS** + $\rightarrow$ b-IHCA GS** + (CRP–) $\rightarrow$ b-HCA All markers– $\rightarrow$ UHCA

In the absence of typical routine histological criteria for H-HCA or IHCA, the other HCA subtypes are likely *include b-IHCA (GS is mandatory to differentiate IHCA and b-IHCA).

**Perform in addition β-catenin staining: aberrant nuclear staining confirm the diagnosis; its absence does not rule out; however, the diagnosis, particularly on needle biopsies, needs molecular biology for definite diagnosis.

3.2.2. Group B (5 b-HCAs and 4 UHCAs). Among the 9 patients, 8 were correctly classified. One UHCA case was classified IHCA.



(a) (b)

Figure 5: Unclassified HCA (UHCA)—(a): this nodule does not exhibit particular features on H&E; (b): nearly diffuse positivity of CD34 immunostaining.

The agreement between routine technics combined to GS compared to the all sets of IHC markers for the identification of the 2 major HCA subtypes gave a kappa index of 0.89, 0.91/0.54 (group A), and 0.92 (group B) ($P < 0.01$) for H-HCA, IHCA/b-IHCA, and b-HCA/UHCA respectively, indicating a good agreement between them.

4. Discussion

Classification of HCA subtypes should allow the selection of patients at high risk of malignant transformation [7–10]. Specific immunohistochemistry is regarded as a main tool to classify HCA in clinical practice [2, 11, 12] and still remains the best method to make the differential diagnosis between FNH and HCA [5, 6]; however, it may not be widely available due to the fact that HCA is a rare and essentially benign disease.

The present study demonstrates the value of routine histology to classify the 2 major HCA subtypes: H-HCA and IHCA (Table 2), which represents more than 80% of cases at least in Europe, without the use of specific IHC. The diagnosis of H-HCA was often easy on routine H&E due to the presence of fat, a sign often used on MRI to identify this subtype [13–16]. The major criterion to identify IHCA was inflammation associated with pseudo-portal tracts containing thick-walled arteries, with frequent ductular reaction [5, 6]. Sinusoidal dilatation, a major criteria used by radiologist to identify IHCA was less sensitive than inflammation to diagnose IHCA. Interestingly enough, it was not possible to identify b-IHCA in the absence of GS staining. Overall, it was possible to strongly suspect an H-HCA and IHCA/b-IHCA on standard routine staining combined to GS in more than 90% of cases related to their hallmark features (Figures 3(a) and 4(a)) and by default the group b-HCA/UHCA. Interestingly enough, it was also possible to distinguish b-HCA from UHCA.

Moreover, this study outlines the limits of routine histology to identify with certainty H-HCA and IHCA (including b-IHCA). First, in H-HCA, steatosis can be mild, focal, or rarely absent, and furthermore areas of sinusoidal dilatation can exist. On the other hand, in IHCA and b-IHCA, sinusoidal dilatation can be mild, focal, or absent; inflammation limited to few portal tracts-like and steatosis—generally focal—could be severe. In addition, in these 2 main subgroups, as well as in all cases of HCA, necrotic areas may lead to remodeling and more or less misleading features. In IHCA particularly, fibrotic bands associated with inflammation and ductular reaction may mimic FNH on routine histology. Additional IHC is mandatory to assess the right diagnosis. The fact that in the control set, we missed 6 out of 10 b-IHCAs based on IHCA criteria raises the question of the similarity between the 2 entities. The number of b-IHCA was, however, too small to be entirely sure to draw any firm conclusion.

If routine histology represents a reasonable means to identify the 2 major subtypes for pathologists with no access to specific IHC, the lack of identification of b-IHCA remains a serious limitation. Interestingly enough, this study demonstrates the impossibility to identify b-IHCA using standard histology. Surprisingly, none of the b-IHCA in our series (test and validation) had cytological or architectural abnormalities such as rosette formation as previously mentioned in b-HCA [3]. Abnormal GS expression is a very useful marker to orientate towards β-catenin-activated HCA (inflammatory or not), particularly when the staining is strong, diffuse, or patchy, even in the absence of aberrant β-catenin nuclear staining. However, GS reading is not always easy as underlined above, particularly when GS staining is faint and focal; in these cases, the distinction between a truly abnormal staining and, a few positive cells around veins in the nodule is hard to make. Indeed, this was the case in rare H-HCA. Cases of β-catenin activation could be observed in H-HCA, but this seems extremely rare. Therefore, the main issue remains the differential diagnosis between IHCA and b-IHCA. In these difficult cases, if there is no nuclear β-catenin staining, the issue can be solved only by molecular biology.

More generally speaking, in the absence of specific complete set of IHC stainings, routine histology combined with GS can be considered as a reasonable surrogate approach for the identification of the 2 major HCA subgroups. In addition, clinical, biological, and radiological data can help to make the right diagnosis of HCA subtype [17, 18].

We have to remember that the very peculiar pattern of GS immunostaining in FNH is particularly helpful for their diagnosis mainly when different types of nodules are associated with the same liver. Finally, in the present work, we acknowledged that the H-HCA and IHCA/b-IHCA subtypes using routine histology were obtained on surgical specimens. In a recent article, we demonstrate that typical H-HCA can be

recognized on biopsy on H&E staining; this is also possible for IHCA, but the % of correct diagnosis is lower [19]. Therefore, to avoid the difficulty of interpretation and the higher risk of errors on biopsies, IHC is highly recommended. IHC not only offers the possibility to identify HCA subtypes, but also to confidently make the differential diagnosis between FNH and HCA [19].

5. Conclusion

The diagnosis of HCA may be difficult particularly for the pathologists are not familiar with these rare tumors. Characteristic features may exist which can be recognized by general pathologists on routine histology, allowing a good estimation of the 2 major HCA subtypes (>80%). Therefore, a "palliative" diagnosis approach can be proposed, when the panel of the antibodies is not available. However, in absence of these features, IHC remains the gold standard to assess the diagnosis of HCA and its subtype. GS is essential to identify β-catenin-activated HCA (inflammatory or not) that is particularly important to detect patient with higher risk of malignant transformation.

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Review Article

Molecular Classification of Hepatocellular Adenomas

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Hepatocellular adenomas (HCAs) are benign tumors developed in normal liver most frequently in women before menopause. HCAs lead to diagnostic pitfalls and several difficulties to assess the risk of malignant transformation in these young patients. Recent advances in basic knowledge have revealed a molecular classification related to risk factors, pathological features, and risk of transformation in hepatocellular carcinoma. Three major molecular pathways have been identified altered in specific HCA subgroups that are defined by either (1) inactivation of hepatocyte nuclear factor 1A (*HNF1A*) transcription factor, (2) activation of the WNT/β-catenin by *CTNNB1* mutations, or (3) activation of the IL6/STAT3 pathway by somatic mutation of *IL6ST*, *GNAS*, or *STAT3*. Here, we will review the different molecular classes of HCA.

1. Introduction

Hepatocellular tumors deriving from monoclonal proliferation of hepatocytes are classically divided in benign hepatocellular adenoma (HCA) and malignant hepatocellular carcinoma (HCC). HCAs are rare tumors most frequently-developed in women before menoaupose and after a long-term use of oral contraception [1]. Other risk factors such as glycogen storage diseases and androgen intake are also classically associated with HCA development. HCA could be complicated frequently by hemorrhage and more rarely by malignant transformation in HCC [2, 3]. For a long time, HCA was considered as a benign monoclonal proliferation of hepatocytes driven by oestrogen exposition [4, 5]. However, molecular classification has redrawn the physiopathological and clinical landscape of HCA [6]. This new classification linked specific risk factor, clinical history, and histological features to each molecular subgroup of HCA [6–9]. In addition, this genotype/phenotype classification has been validated by several groups worldwide demonstrating its robustness and its wide reproductibility in clinical practice [10–15]. In this paper we aimed to describe how genomic analyses enabled us to identify the different HCA molecular subgroups and their specific molecular defects.

2. Molecular Classification of Hepatocellular Adenomas

2.1. Adenomas Inactivated for HNF1A (H-HCA). In 2002, we identified *HNF1A*, as the first driver gene inactivated by mutation in hepatocellular adenomas [16]. *HNF1A* codes for the hepatocyte nuclear factor 1 A, a transcription factor involved in hepatocyte differentiation and metabolism control [17]. Previously, in 1996, Yamagata and collaborators had identified germline mutations of *HNF1A* as the causal alteration of the specific diabetes named MODY 3 for maturity onset diabetes of the young type 3. In MODY3 patients, one allele of *HNF1A* is inactivated in all cells of the organism showing the pivotal role of *HNF1A* partial inactivation in glucose homeostasis dysregulation [18]. In HCA tumor cells, we described complete *HNF1A* inactivation by mutation of both alleles in 35% to 45% of the cases (Table 1) [16]. In most of the cases, both mutations occurred in tumor cells and were of somatic origin. However, in 10% of HCA inactivated for *HNF1A*, one mutation was germline, and consequently, we identified MODY3 patients developing HCA [19, 20]. These patients could also have adenomatosis, a rare condition defined of more than 10 adenomas in the liver [21, 22]. In this line, these results have revealed for the first time

Table 1: Genotype/phenotype classification of hepatocellular adenomas.

Group	%	Genetic alteration		Pathway dysregulated	mRNA markers	Protein markers	Clinical association	Histological phenotype
HNF1A-mutated HCA	30–45%	*HNF1A*	Tumor suppressor gene	Activation of glycolysis, fatty acid synthesis, and mTor pathway	Decrease LFABP1 UGT2B7	lack of LFABP1 expression	Adenomatosis and association with MODY 3 diabetes (*HNF1A* germline mutation)	Diffuse steatosis
CTNNB1-mutated HCA*	10–15%	*CTNNB1*	Oncogene	Activation of Wnt/catenin pathway	Increase GLUL LGR5	overexpression of glutamine synthase and nuclear β-catenin	Risk of malignant transformation Male	Cell atypia and cholestasis
Inflammatory HCA*	40–55%	*IL6ST* (65%) *STAT3* (6%) *GNAS* (5%) Unknown (24%)	Oncogene	Activation of JAK/STAT pathway ("oncogene-induced inflammation")	Increase SAA CRP	SAA and CRP over-expression	Obesity and high alcohol intake Inflammatory syndrome	Inflammatory infiltrate Sinusoidal dilatation Dystrophic arteries
Unclassified	10%	Unknown						

* 50% of *CTNNB1*-mutated adenomas are also inflammatory.

HNF1A as a tumor suppressor gene in addition to its role in metabolism regulation. We further showed that *HNF1A* inactivation induces in hepatocyte dramatic alteration in metabolic pathways and epithelial-mesenchymal transition that can participate to tumor development [23, 24].

In addition the of environmental factor and germline *HNF1A*-mutations, others genetic features could predispose to the occurrence of *HNF1A* mutated adenomas. In this line, we identified heterozygous germline mutations of *CYP1B1* in a subset of patients with H-HCA [25]. All patients with these mutations have a decrease enzymatic activity of the cytochrome p450 *CYP1B1*. Because CYP1B1 is involved in the metabolism of estrogens, it suggests that development of H-HCA could be promoted by a defect in this pathway in relation with exposure to oral contraception.

At the pathological level, HCA with *HNF1A* biallelic mutations exhibited typical features. They are characterized by diffuse steatosis in tumor hepatocytes [6]. We further showed that the homogeneous accumulation of lipids in tumor hepatocytes was related to an increase of fatty acid synthesis induced by *HNF1A* inactivation [26]. H-HCA can be easily diagnosed using pathological examination because these adenomas are characterized by a constant and specific lack of FABP1 expression in the tumor hepatocytes [12, 27].

2.2. β-Catenin Activated Adenomas (β-HCA). The Wnt/catenin pathway is a pivotal oncogenic pathway involved in solid and haematopoietic tumors. *CTNNB1,* the gene coding for β-catenin, is activated by somatic mutation in a large number of different tumor types like medulloblastoma or breast cancer [28]. Moreover, it is the most frequently mutated oncogene in hepatocellular carcinoma (from 20 to 40% of the cases) [29]. *CTNNB1*-activating mutations target few serine and threonine amino acids in β-catenin, residues that are physiologically phosphorylated by the APC/GSK3/axin complex inducing degradation of β-catenin by the proteasome. *CTNNB1* mutations impaired the phosphorylation by the APC/GSK3B/AXIN complex and led to the translocation of β-catenin into the nucleus [28, 30]. In this condition, the oncogenic effect of β-catenin is fully active [31, 32].

Mutations activating β-catenin are described in 10 to 15% of HCA (Table 1) [6, 33]. Male are overrepresented in this subgroup of HCA [34]. Furthermore, β-HCA are often characterized by pseudoglandular formation, cell atypia, and cholestasis at the pathological level. Using immunochemistry, we showed that β-HCAs are characterized by a strong cytoplasmic expression of glutamine synthase and nuclear expression of β-catenin in tumor hepatocytes. However, despite a good specificity, these markers have a lack of sensitivity for the diagnosis of β-HCAs and HCA should be screened for *CTNNB1* mutations [12, 27, 35, 36], when glutamine synthase and β-catenin markers are not informative.

Importantly, we showed that HCA with activating mutations of β-catenin have a high risk of malignant transformation in HCC [6, 36, 37]. Moreover, distinguishing HCA from well-differentiated HCC developed on normal liver could be challenging. Consequently, all HCA harboring a mutation of β-catenin should be surgically resected in order to avoid the risk of malignant transformation. In this context, the performance of immunohistochemical marker developed to discriminate high-grade dysplastic nodules from very early HCC (like glutamine synthase, glypican 3 or hsp70) on cirrhosis remains poorly explored to differentiate HCA from very well differenciated HCC on normal liver and should be used with caution [38]. A recent study has shown that the combination of glypican 3 and HSP70 has a good specificity (100%) but an insufficient sensitivity (43%) to distinguish HCA from well-differenciated HCC [38, 39]. However, the small numbers of tumors analyzed preclude the generalization of these markers in clinical practice and required additional studies. Another concept is that some hepatocellular tumors will remain borderline tumors between HCA and HCC despite histological analysis by an expert pathologist. In this grey zone, *CTNNB1* mutations are also overrepresented [6, 34].

In this line, screening for *CTNNB1* mutation should be mandatory to detect HCA with a potent risk of malignant transformation and borderline lesion between HCA and HCC that should be resected.

2.3. Inflammatory Adenomas (IHCAs). In the physiological point of view, the most important breakthrough has been performed by the identification of the so-called "inflammatory HCA" and dissection of IL6/JAK/STAT pathway [40, 41].

IHCAs are characterized by the activation of JAK/STAT and interferon I and II pathway [40, 42]. This subtype of adenomas exhibited strong pathological hallmark: inflammatory infiltrates, dystrophic arteries, and sinusoidal dilatation [43]. Immunohistochemical marker could be used as diagnostic tool for this subtype of HCA. Inflammatory HCA exhibited a cytoplasmic overexpression of SAA and CRP, two proteins of the acute phase of inflammation, in the tumor hepatocytes (Table 1) [12, 15]. Sometimes, IHCAs are associated with inflammatory syndrome and related anemia [44]. Peripheral inflammatory syndrome can regress after resection of the tumor, and it could be considered as a "paraneoplastic syndrome" [45, 46]. IHCA occurred more frequently in patients with high alcohol consumption and obesity, two conditions associated with chronic cytokine production [6, 46]. We also described an IHCA transformed in HCC mutated for both gp130 (*IL6ST*) and β-catenin (*CTNNB1*) and developed on the background of Castleman disease [47]. In this rare disease, a chronic IL6 systemic secretion is produced by a lymphoproliferative disorder. It underlined again the possible role of chronic cytokine production (obesity, high alcohol consumption, and Castleman disease) as a predisposing factor to inflammatory HCA occurrence. Recently, we deciphered the molecular alterations leading to the activation of inflammatory pathway in the tumor hepatocytes.

We described the oncogenes that explain the hepatocytes proliferation and the inflammatory phenotype ("oncogene-induced inflammation"). The most preeminent oncogene identified was gp130 (*IL6ST*) [42]. 65% of inflammatory HCAs exhibit a somatic activating mutation of gp130. Gp130

is the coreceptor of IL6R. Activating mutations of gp130 led to the constitutive activation of the JAK/STAT pathway in the absence of the IL6 ligand [42, 48]. A small subset of HCC exhibited both gp130 and β-catenin-activating mutations. Interestingly, these HCC are developed in normal liver and could be derived from HCA.

We also described for the first time in human tumors somatic mutations activating *STAT3* [49]. These mutations explained the uncontrolled activation of JAK/STAT pathway and the observed phenotype in 6% of the IHCA. Finally, we discovered *GNAS*-activating mutations in 5% of inflammatory HCA [50]. *GNAS* gene coded for alpha subunit of Gs protein and is a well-known oncogene in pituitary and thyroid adenomas. Mutations of *GNAS* gene impaired the GTPase activity of alpha subunit and led to its permanent activation by an unregulated binding of GTP. As a consequence, cyclic Amp accumulates in the cells [51]. In adenoma, we described a crosstalk between cyclic Amp and JAK/STAT pathway that explained the mild inflammatory phenotype in *GNAS*-mutated HCA [50]. In this line, we also described HCA in patients with McCune Albright syndrome [52]. McCune-Albright syndrome is an orphan disease due to somatic postzygotic mosaic *GNAS* mutation. This genetic disorder is characterized by pituitary and thyroid adenomas, fibrous bone dysplasia, and "café au lait" skin macula [51]. Consequently, McCune Albright syndrome also predisposed to HCA development.

2.4. Unclassified Adenomas. Finally, 10% of HCAs have no known genetic alterations or specific histological phenotype (Table 1) [34]. The molecular drivers of this subtype of HCA remain to be determined.

3. Mechanism of Development of Hepatocellular Adenomas: A Contribution of Different Genes with a Genotoxic Signature

In the canonical point of view, malignant hepatocellular tumors (HCC) arise on chronic liver disease, mainly cirrhosis or chronic HBV infection, whereas hepatocellular benign tumors are developed on normal liver. However, several clinical, pathological, and molecular observations have challenged these dogmas. First, HCC could develop on normal liver, and predisposing genetic factor and genetic drivers involved in tumor initiation remain poorly described [53]. A simple clinical observation supports the fact that HCA is not a stochastic and isolated tumorgenic event: 40% of patients with HCA have multiple HCA in the liver suggesting an individual predisposition to develop this rare disease [34]. Also, several genetic disease and environmental factors favored hepatocytes proliferation and benign tumors initiation. Moreover, since several decades, the major HCA risk factors, oestrogen and androgen consumptions, have been identified as classical genotoxins [54–56]. Association between estrogen exposure and HCA occurrence was first described in the seventies when oral contraception was of widespread use in western countries [4, 55, 57]. In addition, tumor regressions after estrogen withdrawal have been reported [56]. It underlined that HCA is a hormonal-driven benign tumor. Nevertheless, estrogen exposure due to oral contraception is frequent, but HCA occurrence is rare (around 3/100,000) [55]. It seems that others genetic and/or environmental factors are required to promote HCA development. More recently, the use of a third generation of oral contraceptive with lower dose of estrogen could have modified the epidemiology of HCA [58]. However, robust epidemiological data comparing these two periods in western countries are lacking. In addition, the incidence of HCA in eastern countries, where oral contraception is not frequently used, remains to be evaluated. Differences in incidence and molecular subtypes of HCA between eastern and western countries could help to understand the role of estrogen exposure and other risk factors like obesity and alcohol consumption in the development of benign liver tumors [46, 59]. When we analyzed the spectrum of mutations of *HNF1A* in HCA, we also showed that *HNF1A* somatic mutations were frequently caused by G to T transversion suggesting a genotoxic exposure at the origin of the mutations [60]. Causes of this genotoxic signature remain to be elucidated, and the role of oestrogen exposition in this genotoxic damage should be further analyzed. A hypothesis is that HCA development could be favored by both a genetic predisposition in combination with an exposure to different genotoxic agents.

In this line, predisposing genetic factors like *HNF1A* germline mutation related to MODY3 diabetes and *GNAS* mosaic somatic mutations related to McCune Albright disease are strong risk factors of adenomas occurrence [19, 50]. Moreover, patients with glycogenosis type IA defined by germline inactivating mutation of glucose-6 phosphatase have a huge risk to develop multiple HCA during their followup [61–63]. All these data underlined that hepatocellular benign tumors are often developed on a predisposing abnormal liver background. This hypothesis could be called "benign tumorigenic field effect" as a mirror of the "carcinogenic field effect" described for HCC developed on cirrhosis. The "benign tumorigenic field effect" is a conjunction between genetic (*HNF1A* germline mutation, *GNAS* mosaic postzygotic mutation, and others unknown modifier genes) and environmental factors (oestrogen and androgen expositions) [20, 56, 57, 60, 64]. In addition, we showed a role of CYP1B1, a cytochrome p450 unit involved in detoxification of catechol estrogens, in the occurrence of HCA [25]. We identified a germline *CYP1B1*-inactivating mutation in 12.5% of patients developing *HNF1A*-inactivated HCA. Moreover, when analyzing the spectrum of somatic mutations in *HNF1A*, we identified a genotoxic signature typical of molecule inducing adduct to DNA at guanine [60]. Thus, a combined genetic predisposition and genotoxic effect could explain the frequent occurrence of multiple HCA in the same patient, and despite that the surrounding nontumor liver appears to be mainly "histologically (sub)normal," the liver is tumorigenic.

4. Conclusion

A long path has been walked in the area of hepatocellular benign tumors since Edmonson described the association

between HCA and oral contraception [4]. Now, the discovery of genetic drivers of HCA has refined our knowledge of the life history of HCA from risk factors and clinical features to the risk of malignant transformation. However, several goals are still unmeet. First, the risk factors leading to HCA development are partially understood. Most of the patients have no known genetic factors predisposing to HCA occurrence. Moreover, all patients with genetic alterations predisposing to HCA will not develop tumors. So, additional genetics and environmental factors remain to be discovered. Thus, in addition to activating mutations of β-catenin, other genetic alterations leading to full malignant transformation have to be deciphered. Finally, several driver genes of benign tumorigenesis are still unknown, especially in the group of inflammatory HCA without known driver mutations and unclassified HCA. Ultimately, these genetic alterations will constitute therapeutic target for biotherapy that will be used in unresectable HCA or in other malignancies harboring the same genetic events.

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Review Article

The Management of Pregnancy in Women with Hepatocellular Adenoma: A Plea for an Individualized Approach

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Because of the risk of hormone-induced growth and spontaneous rupture of hepatocellular adenoma (HCA) during pregnancy, special considerations are required. Due to the scarcity of cases, there is no evidence-based algorithm for the evaluation and management of HCA during pregnancy. We think it should be questioned if it is justified to discourage pregnancy in all women with HCA. The biological behavior of this benign lesion might be less threatening than presumed and a negative advice concerning pregnancy has great impact on the lives of these young female patients. The balance between the pros and cons of hepatic adenomas and pregnancy should be reconsidered. In our center, pregnancy in women with an HCA up to 5 cm is no longer discouraged in close consultation with the patient, her partner, and members of the liver expert team.

A strong association between hepatocellular adenoma (HCA) and the use of oral contraceptives (OC) was first described in 1973 [1]. The hypothesis that there is a relation between steroids and HCA has been supported by many authors but is still not understood [2–4]. Due to the increased levels of endogenous hormone production, which may cause hormone-induced growth and rupture, HCA requires special attention during pregnancy [5, 6]. Patients with a growing or ruptured HCA mostly present themselves with persistent or acute severe pain localized in the upper right quadrant and in the epigastric region. In the literature, the maternal and fetal mortality risks of ruptured HCA during pregnancy has been reported to be 44 and 38% respectively [7]. However, all these cases were published in the 1970s or 1980s, in which there might have been a delay in diagnosis as the entity of ruptured HCA was not well known and less advanced imaging methods were used.

In the recent years the widespread use of highly advanced image modalities has probably decreased the delay in the diagnosis of HCA and the associated maternal and fetal mortality significantly. Because of the unpredictable behavior of HCA during the increased levels of endogenous hormones, we used to advise women with a large HCA or a growing and hormone-sensitive HCA to avoid pregnancy, as most other experts in this field do [6, 8]. Even if HCA was incidental findings previous to a pregnancy without having caused any complications, women were still advised not to get pregnant as long as the HCA is present. Because of the overall agreed advice to avoid pregnancy in patients with HCA, the diagnosis of HCA has severe impact on the lives of these young fertile women.

As to date, there are limited data about the behavior of HCA during pregnancy and labor.

From the international literature between 1966 and 2003, Cobey and Salem retrieved 26 cases of women presenting with HCA during pregnancy or early postpartum and proposed an algorithm for their diagnosis and management [7]. Presentation was acute and often dramatic with rupture of the adenoma in 16 women and frequently with a delay in establishing the correct diagnosis, with high maternal and fetal mortality (44% and 38%, resp.). The hormone-induced growth and risk of rupture seemed to be the highest during the third trimester of pregnancy, most probably because of the cumulating level of estrogens and an increase in

the hyperdynamic circulation combined with an increase in vascularity of the liver [7]. An aggressive approach towards resection of HCA was advocated, especially for those greater than 5 cm. Small adenomas were supposed to be managed by observation [7]. It is important to realize that most of these reports were published in a time period during which this disease entity was relatively unknown and treatment in an emergency setting was less advanced.

In our hospital, we monitored 12 women with one or more documented HCAs during a total of 17 pregnancies. In four cases, HCA grew during pregnancy, requiring a Caesarean section in one patient (two pregnancies) and RFA in one patient during the first trimester of pregnancy because of significant growth of the adenoma. All pregnancies had an uneventful course with a successful maternal and fetal outcome [9]. We concluded not to discourage all women with HCA from pregnancy. In our tertiary referral center, we closely observe pregnant women with a HCA smaller than 5 cm in a clinical trial [10]. In this study, the size of the lesion is an exclusion criterion when exceeding 5 cm, but the number of HCAs present in the liver is not. Three studies investigated the association between the risk of rupture and the number of HCAs [11]. This risk did not differ between single and multiple HCAs [12–14]. In our previous study, the number of HCAs in the women observed during pregnancy varied between 1 and more than 10 HCAs. We concluded that only in women with large tumors and a complicated pregnancy previously, pregnancy should be discouraged [9].

Furthermore, in our opinion, none of the subgroups from the molecular and pathological subtype classification of the Bordeaux group legitimizes objection against pregnancy. Although the number of cases described in literature is small, no difference has been demonstrated in the risk of bleeding between the two major subgroups, the inflammatory and the hepatocyte nuclear factor 1α-inactivated HCAs [15, 16].

If women have large tumors or have experienced complications of HCA in previous pregnancies, an intervention (surgery, RFA, embolisation) should be recommended before pregnancy. Moreover, in 2006 we reported a series of 48 patients in which 44% of HCA were discovered after the patient had sustained at least one pregnancy [17].

Intervention during pregnancy may be associated with greater risk for both mother and child. The Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) provided guidelines for diagnosis, treatment, and use of laparoscopy for surgical problems during pregnancy [18, 19]. In one in 635 pregnancies, a nonobstetric operation, in particular appendectomy, cholecystectomy, and adnexal procedures, is required during pregnancy [20]. These guidelines suggest that the laparoscopic approach should be preferred instead of laparotomy in most abdominal operations.

The maternal and fetal outcomes following abdominal surgery in pregnancy improved over last decade but the exact risk of HCA-related interventions during pregnancy to both mother and fetus is unknown [21]. Abdominal surgery may be more difficult during pregnancy in the late second and third trimester because of the limited wideness in the upper abdomen due to the enlarged uterus and risk of steatotic changes of the liver in these patients. General anesthesia seems to have the least risk in the 2nd trimester of pregnancy [5]. The role of RFA during pregnancy is not yet been studied extensively. In our previous study, we described a RFA procedure during the first trimester of pregnancy [9] and a pregnant patient with a HCA which was treated by RFA during her second trimester of pregnancy (18th week of gestation) was reported by Fujita et al. [22]. After systematically reviewing the literature, Wilson et al. suggested that angioembolization and formal resection in case of hemorrhage of HCA during pregnancy is safe for both the mother and the fetus with good clinical outcomes [23]. We believe that selective arterial embolization should only be used as a live-saving treatment in those cases where RFA or surgery is inadequate or too risky to control the bleeding adenoma. The increased risk of radiation exposure to the fetus, especially before 26 weeks of gestation [24, 25], should be avoided if possible.

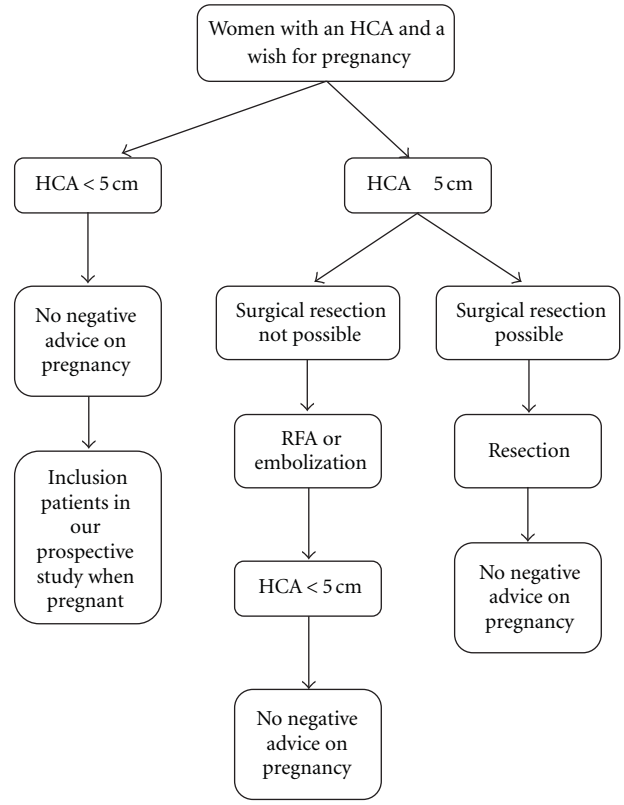

Figure 1: Flowchart for women with a HCA and a wish for pregnancy.

Because HCA might have the tendency to rupture during delivery, some authors suggest a Caesarean section (C-section). In our study three C-sections (two patients) were performed, without complications. In one case the C-section was performed in consultation with the patient because of marked growth and an unknown risk of rupture of the HCAs. In the other, C-section was due to decelerations on the cardiotocography [9]. All other patients had a normal delivery without complications. Therefore, in our opinion patients with HCA may deliver vaginally if there are no complicating factors, like perinatal problems.

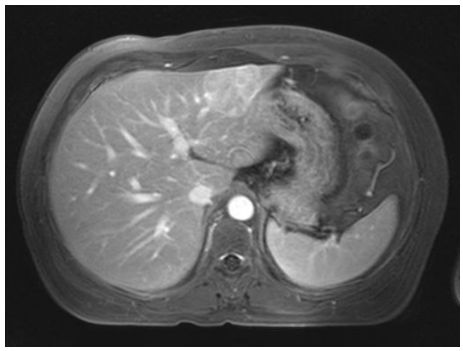

Figure 2: An example of a woman with a HCA of 4.2 cm in segment 2/3 in which pregnancy will not be discouraged.

In conclusion, it seems to be justified that a pregnancy should be discouraged in patients with a large HCA (>5 cm) or those who experienced complications of the lesion in previous pregnancies (Figure 1). In those cases a surgical resection, RFA, or embolisation should be recommended before pregnancy. In our center we do not discourage pregnancy in women with a HCA <5 cm (Figure 2) if they accept the risk of interventions in case of growth of the adenoma. Close guidance of these women and monitoring of the hepatic adenoma by liver ultrasound every 6 weeks during pregnancy are strongly advocated [10, 26].

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Research Article

How Normal Is the Liver in Which the Inflammatory Type Hepatocellular Adenoma Develops?

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The inflammatory type hepatocellular adenoma (IHCA) is a subtype of HCA which is a benign liver tumor, predominantly occurring in young women in an otherwise normal liver. IHCA contains either a mutation of gp130 or STAT3. Both mutations lead to a similar morphologic phenotype and to increased expression of C-reactive protein (CRP) and/or serum amyloid-A (SAA). IHCA comprised about 40% of all HCAs and is associated with obesity. We investigated the histomorphological and immunophenotypical changes of the nontumorous liver of 32 resected IHCA specimens. Similar types of changes are present in samples taken adjacent to tumor and distant ones. The lobular architecture is well preserved. Mild/moderate steatosis is found in a high frequency which is in accordance with the median BMI of 32 in our cases. Of note are the regular findings of sinusoidal dilatation, single arteries, and minute CRP foci which are all features of HCA. These distinct CRP foci are mostly found in cases of multiple IHCA which indicates that the remnant liver may also contain IHCA foci. These findings show that the nonlesional liver in IHCA does contain abnormalities, and this may have consequences for the followup, especially since it is known that obesity may stimulate malignant growth.

1. Introduction

Hepatocellular adenoma (HCA) is a benign primary hepatocellular tumor, occurring predominantly in females in their reproductive age and is associated with long-term use of oral contraceptives [1, 2]. Recently, a rising incidence has been reported, partly due to improved application of diagnostic imaging techniques, for example, CT, MRI [3]. HCA is divided into 3 subgroups according to 3 different genetic mutations: hepatocellular nuclear factor-1α (HNF1α) gene-mutated type HCA, β-catenin gene-mutated type HCA, and inflammatory type HCA (IHCA) which contains a somatic mutation of IL6ST gene. The latter mutation, encoding gp130, is found in 60% of IHCA, and a somatic mutation of STAT3 gene is found in 12% of IHCA [4, 5]. A fourth group represents HCA without any of these mutations. Of note, the IHCA may concurrently contain β-catenin mutation which increases the risk of malignant transformation. The HCA subtyping can be performed by visualizing the coded proteins of the mutated genes by immunohistology [6–8]. HCA containing HNF1α mutation shows absence of liver fatty acid binding protein-1 (LFABP-1) in contrast with the diffuse hepatocytic expression of this protein in normal livers. IHCAs, both those with IL6ST mutation and STAT3 gene mutation, show increased C-reactive protein (CRP) and/or serum amyloid-A (SAA) expression [5]. HCA containing β-catenin mutation shows nuclear translocation of β-catenin expression, but this finding may be focal and patchy, whereas an aberrant diffuse expression of glutamine synthetase (GS) is also indicative of β-catenin mutation [6–8].

IHCA represents the largest subgroup of HCA and has been reported to be related with systemic disorders, such as obesity, metabolic syndrome, and alcohol abuse [7, 9]. One report mentioned that IHCA patients with a high body mass index (BMI ≥ 25) represent 60% of their study group in which the mean BMI is 28 [9]. Subgroups of HCA except the β-catenin gene-mutated type rarely show malignant transformation into hepatocellular carcinoma (HCC) although a recent study reported an increased risk in HCA occurring in overweight or obese male patients [10]. These findings suggest that in obese individuals the whole hepatic microenvironment is influenced by systemic factors that may favor tumor development, in accordance with the postulation that obesity increases the risk of cancer development [11].

In the present study, we investigated the histological features of the nontumorous liver parts of 32 resected IHCA specimens, to gain insight in the hepatic microenvironment in which IHCA develops, also because IHCA are often multifocal. Therefore, knowledge about the nonlesional liver tissue that corresponds to the remnant liver after tumor resection may influence the followup management of IHCA patients.

We found that although the lobular architecture is largely well preserved, the nontumorous liver frequently shares several abnormal features with the adenoma, such as sinusoidal dilatation and single arteries. Moreover, many cases also contain several foci of minute HCA-like areas with focal increase of CRP/SAA. These findings suggest that the nonlesional part of HCA-containing livers harbors changes that may potentially stimulate adenomatous growth. This is especially true for livers with multiple adenomas.

2. Patients and Methods

2.1. Patients. Thirty two patients, all of them females (mean age 33.5 ± 8.8 years), who underwent partial liver resection for IHCA, were included. Cases were selected on the availability of sufficient amount of adjacent nontumorous liver (AL) and/or distant nonlesional liver tissue (DL). The latter sample was taken at least 3 cm distant from the tumor.

2.2. Histology. A representative slide of the transformation area of tumor and adjacent nontumorous liver tissue (AL, $n = 32$) and one sample from the distant nonlesional part (DL, $n = 22$) were reviewed without knowledge of clinical data and the features of the corresponding tumor. Slides were stained with hematoxylin-eosin (HE) and Masson trichrome. The AL and DL samples were assessed separately for the following features: liver architecture, steatosis, steatohepatitis, sinusoidal dilation, single artery, and ductular reaction. Grading of steatosis and steatohepatitis was performed according to the scoring system for nonalcoholic steatohepatitis (NASH) proposed by Brunt et al. [12]. In summary, steatosis: 0 = absent; 1 = steatosis observed in up to 33%; 2 = more than 33% and less than 66%; 3 = more than or equal to 66% and steatohepatitis: 0 = absent; 1 = occasional ballooned hepatocytes, mild portal chronic inflammation; 2 = obvious ballooned hepatocytes, portal and intra-acinar chronic inflammation noted, mild to moderate; 3 = ballooning and disarray obvious with mild chronic inflammation, portal chronic inflammation, mild or moderate.

Grading of sinusoidal dilation followed the criteria mentioned by Rubbia-Brandt et al. [13]. Sinusoidal dilation: 0 = absent; 1 = centrilobular involvement limited to one-third of lobular surface; 2 = two-thirds lobular surface involved; 3 = complete lobular surface involved. Liver architecture is scored as preserved (1) or abnormal (0). Single artery and ductular reactions (DRs) are described as absent (0) or present (1). Single arteries are defined as arterial structures without accompanying bile duct and/or not localized in a portal tract structure. Assessment of DR is described below.

Table 1: Antibodies applied for immunohistology.

Antibody	Dilution	Retrieval methods	Company
β-catenin	1 : 100	Tris-EDTA	BD Transduction (USA)
GS	1 : 4000	Tris-EDTA	Millipore (USA)
CRP	1 : 200	Tris-EDTA	Abcam (UK)
SAA	1 : 200	Protease 8 min	Dako (DK)
CK19	1 : 100	Protease 12 min	BD Bioscience (USA)
CD34	1 : 20	Tris-EDTA	Dako (DK)
α-SMA	1 : 800	Tris-EDTA	Dako (DK)

2.3. Immunohistochemistry. The immunohistological expression of SAA/CRP on tumor tissue was already performed at an earlier, diagnostic stage to establish the diagnosis of IHCA according to the Bordeaux classification [7]. GS and β-catenin staining were also completed at the earlier diagnostic stage to assess possible β-catenin mutation. For the present study, AL and DL samples were stained according to the same protocol, and additional immunostaining with K19, CD34, and α-SMA was performed. K19 increased the feasibility to assess DR as the ductular structures were highlighted by K19 labeling. The presence of 4 or more ductular profiles per portal tract is regarded as the presence of DR [14].

CD34 visualized sinusoidal capillarization and single arteries, whereas α-SMA labeled myofibroblastic transformation of hepatic stellate cells. The antibodies used for the immunohistological staining are mentioned in Table 1 including the applied dilutions and retrieval methods.

3. Results

3.1. Architecture: Generally Well Preserved. In all AL and DL samples, the overall lobular architecture was largely well preserved. A normal distribution pattern of portal tracts and central veins was recognizable. The transition from lesional to nonlesional tissue was usually recognizable by the slightly pushing, irregular border of the nonencapsulated tumor, except in hemorrhagic or necrotic parts where a fibrous scar may have developed and form a capsule. The regular

transitional areas showed smaller, compactly arranged tumor hepatocytes to slightly larger hepatocytes of the nonlesional part, containing more cytoplasm. Portal tracts in these transitional areas frequently contained several thick-walled arteries but otherwise included normal bile ducts and portal veins. Portal inflammatory infiltrates varied but was usually nonconspicuous. Fibrosis was usually absent.

Figure 1 illustrates the several aspects of the transitional area.

3.2. Steatosis: Common Finding. Steatosis was a common finding in the nonlesional liver tissue as it was observed in 23/32 (70%) AL samples and 13/22 (59%) DL ones. The majority of cases showed mild to moderate degrees of steatosis. Severe steatosis is present in 3 AL and 2 DL samples. Steatohepatitis was rare, being present only in 2/32 patients, both in the AL and DL samples.

In the IHCA itself, steatosis was less common than in the nontumorous counterpart. Steatosis was present in 15/32 (47%) tumor samples. In the steatotic liver, based on the steatosis of AL samples, there was a similar frequency of IHCA with (12/23, 52%) and without fatty changes (11/23, 48%) whereas in the nonsteatotic liver most tumors were nonsteatotic (6/9, 67%). A steatotic tumor in a nonsteatotic liver is less common (3/9, 33%). When the frequencies were based on the steatosis of the DL samples, the majority of tumors in the steatotic liver contained fatty changes (8/13, 62%). Similar with the findings of the AL samples, tumors of nonsteatotic DL samples were mostly nonsteatotic as well (6/9, 67%).

Of note, 20 of the 32 patients have high BMI values, leading to a median BMI of 32.55 ± 4.9.

Figures (2(a) and 2(b)) show the steatotic changes in the transitional area and in a DL sample.

3.3. Sinusoidal Dilatation: Frequent Phenomenon. SD was a frequent phenomenon in both AL and DL parts showing a frequency of 59% (19/32 cases) and 77% (17/22 cases), respectively. The areas of dilated sinusoids were of variable extent and rather randomly distributed in the lobules unlike the regular centrilobular punched-out pattern of outflow obstruction. Nevertheless, we have applied the Rubbia-Brandt et al. scoring system [13] that follows the lobular architecture to allow a semiquantitative scoring. In both AL and DL samples, the vast majority of SD was of mild degrees, as observed in 74% and 76% of those cases showing SD. Of the 5 cases with moderate and severe SD in their AL samples, 3 cases showed mild SD in their corresponding DL, 1 case had moderate SD, and 1 case had similarly severe SD in their DL. The 2 latter cases represented 2 of 4 DL cases with moderate and severe SD. The 2 remaining DL cases showed mild SD in their corresponding AL.

3.4. Single Arteries/Arterioles: Regularly Seen Even in Distant Samples. There was a similar frequency of single arteries/arterioles in AL and DL samples. Single arteries were present in 12/32 AL samples (38%) and in 8/22 DL samples (36%). As in the HCA, these single arteries were both present in small groups and as truly single arterial structures in the hepatic lobule (Figures 2(c) and 2(d)).

3.5. Immunohistology: Minute Foci of CRP-Positive Areas; Ubiquitous DR; Activated Myofibroblasts. The expression pattern of GS confirmed the preserved lobular architecture in AL and DL samples as shown by the perivenular distribution of cytoplasmic GS in hepatocytes in the centrilobular areas (Figures 1(a)–1(d)). Bile ductal and ductular cholangiocytes showed a faint blush of cytoplasmic GS expression. A normal membranous β-catenin labeling was present in all hepatocytes but no nuclear expression. Bile ducts and ductules also showed membranous but no nuclear β-catenin expression.

All AL and DL samples showed a normal periportal pattern of CRP expression in hepatocytes. However, in 14/32 cases, minute foci of aberrant CRP expression were observed in the hepatic lobule, consisting of 6 AL samples, 4 DL samples, and 4 other cases of which both the AL and DL samples contained CRP-positive foci. Eleven of these 14 cases concerned livers with multiple adenomas (Figure 3). In the studied group, 21/32 IHCAs were multiple adenomas. None of the minute CRP-positive foci showed GS positivity and/or nuclear β-catenin expression.

Additional immunohistology was performed with K19, CD34, and α-SMA.

DR was practically ubiquitous, being present in 29/32 (91%) AL samples and 21/22 DL (95%) ones (Figure 4(a)).

CD34 staining showed a normal distribution pattern of vascular endothelial labeling and periportal sinusoids. There was no increase of CD34 expression in the rest of the sinusoids. In contrast with CD34, an increased α-SMA expression in the sinusoids was seen in 28/32 (88%) AL and 17/22 DL (77%) (Figure 4(b)). Diffuse increase of α-SMA was seen in 15/28 (54%) of the α-SMA-positive AL samples, and a focal increase localized in the areas of SD was present in 46%. A similar frequency was observed in the DL samples with 9/17 (53%) diffuse distribution and focal increase of α-SMA in 47% of the α-SMA-positive DL samples.

4. Discussion

In contrast with HCC which usually develops in a liver with long-standing chronic liver disease, HCA is mostly found in an otherwise normal liver. IHCA is one of the variants of HCAs representing 40–50% of all HCAs [1]. The 2 different mutational backgrounds of IHCA concerning gp130 and STAT3 lead to a similar morphology and immunophenotype of increased SAA/CRP in the tumor hepatocytes [5].

In the present study, we analyzed the histological and immunophenotypical changes of the nonlesional liver parts of resected IHCA specimens which include samples taken adjacent to the tumor and distant ones. Similar types of histological and immunophenotypical features were found in these two sample types, albeit in variable degrees and frequencies. Among these changes some features represent changes that are also present in IHCA but otherwise not

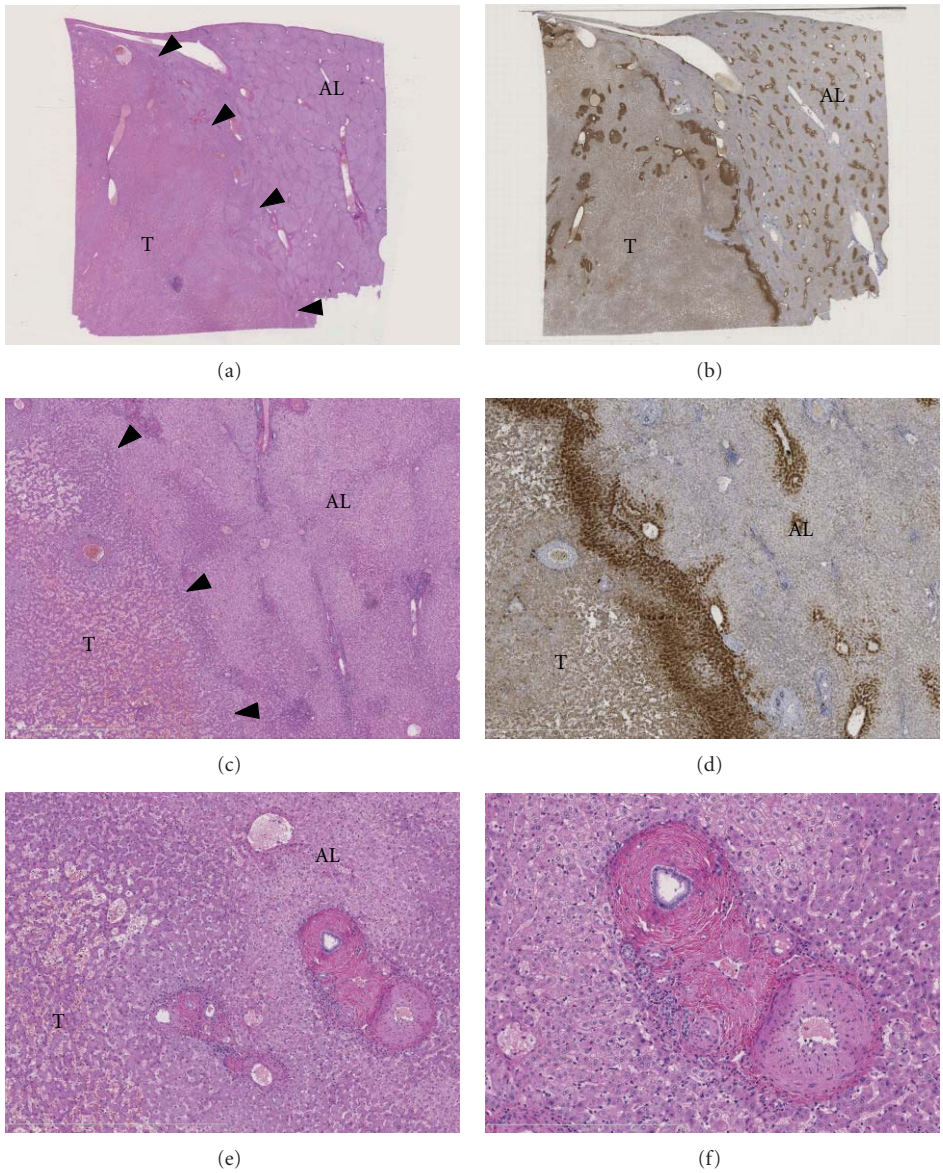


Figure 1: The transitional area of IHCA and adjacent liver. (a, c) HE stained whole slide (a) and detail (c) of a transitional area of an IHCA (T) and the nontumorous adjacent liver (AL). Arrowheads indicate the noncapsulated border of the tumor. (b, d) Glutamine synthetase expression of (a, c) highlights the difference in architecture of IHCA and adjacent liver (AL). In the AL part, glutamine synthetase expression in perivenular areas accentuates the preserved lobular architecture. (e, f) Portal tracts containing thick-walled arteries at the border of tumor (T) and adjacent liver (AL).

found in normal livers, for example, sinusoidal dilatation, single arteries, and foci of CRP-positive hepatocytes.

The lobular architecture is generally well preserved as also confirmed by the normal perivenular distribution pattern of GS expression. Steatosis is very common, being present in 60–70% of the distant and adjacent nonlesional samples, which is in accordance with the high BMI of our study population and in line with the reported relation of IHCA with obesity [7, 9]. Although the latter condition is known to enhance carcinogenesis [11], the tumorigenic role of obesity in IHCA has yet to be elucidated. In the steatotic livers, based on the pattern of the distant samples, almost two-thirds of the tumors contain fatty changes. In the nonsteatotic livers, two-thirds of the tumors are nonsteatotic. Although much higher numbers of patients are necessary for robust conclusions, the above findings indicate that fatty changes in the tumor might be secondary to the fatty constitution of the liver in which the IHCA develops. The scarcity of steatotic tumors in nonsteatotic livers supports this view.

Of note are the vascular abnormalities consisting of sinusoidal dilatation and single arteries. Several types of vascular changes in the nontumorous liver have been described in an early study on telangiectatic focal nodular

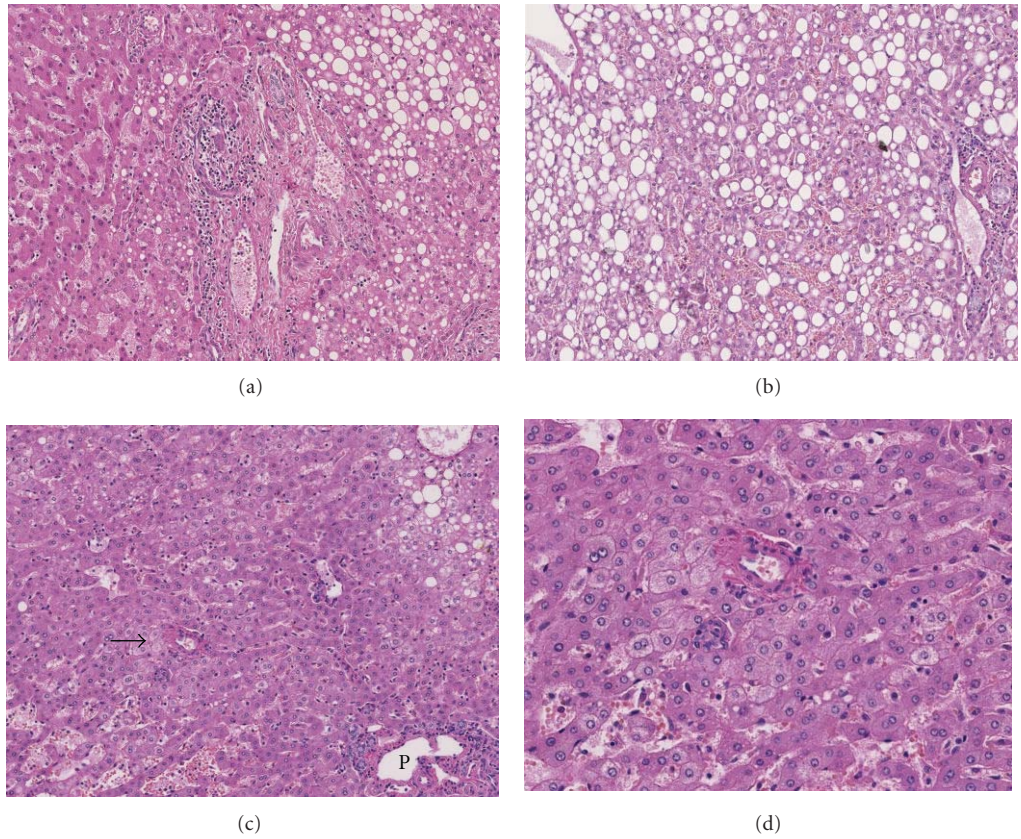


Figure 2: Steatosis and vascular changes. (a) The transitional area of a nonsteatotic adenoma (left part) with a steatotic adjacent nontumorous liver (right part). (b) Moderate steatosis in a distant sample. A portal tract is present in the right lower corner and a central vein in the left upper corner (*). (c) Vascular changes in a distant sample. A portal tract (P) is present in the right lower corner and a central vein in the right upper corner (*). An area with dilated sinusoids is present in the left lower corner. The arrow indicates a group of single arteries. (d) Detail of the single arteries.

hyperplasia which is the obsolete term of IHCA according to the new classification [15]. These features are not found in normal livers, neither do these features belong to the spectrum of changes of fatty liver disease, but these features are characteristics of IHCA. Single arteries are also frequently found in HCC and are even part of the criteria to establish the diagnosis of early HCC [16]. Sinusoidal dilatation appeared to be a common finding in the nonlesional liver parts, both those adjacent to the tumor and the distant samples. The fact that sinusoidal dilatation is also present in the nonlesional tissue indicates a systemic effect. In our study group of women in their reproductive stage, long-term use of oral contraceptives may have a contributory role as these agents are known to cause sinusoidal dilatation and peliotic changes. However, the concurrent presence of sinusoidal dilatation and single arteries in the nonlesional liver parts is suggestive for a common background factor leading to these vascular abnormalities. In HCA, these changes have been related to an increased gene expression of Angiopoietin-1, a vascular growth factor of the Angiopoietin/Tie-2 system [17, 18]. Excess Ang-1 has been reported, both in animal models and *in vitro* to induce vascular remodeling including dilated sinusoids and vessel-forming capacity [19–21]. In another study, we have found increased Ang-1 in HCC [22] in which single arteries are frequently found. These arteries increase in numbers paralleling tumor growth in HCC, and this phenomenon is regarded as tumor angiogenesis as the arteries form the vascular supply of the growing tumor [23]. The fact that there is no obvious tumor growth in the nonlesional liver of our IHCA study group renders angiogenic activity in these parts rather redundant. It is however plausible that an excess of Ang-1 produced by the tumor, may exert its effects in the nonlesional parts. In particular, because Tie-2 receptor which is the specific tyrosine kinase receptor of Ang-1, is ubiquitously present on the sinusoidal endothelial cells and vascular endothelial cells of histologically normal livers [17].

The dilated sinusoids usually lead to variable degrees of atrophy of the hepatic parenchyma. Paralleling these degenerative changes is the increased expression of α-SMA in these areas, reflecting activation of hepatic stellate cells into myofibroblasts. The latter process is probably also induced by steatosis which is present in the majority of cases and which is known to be a potent inducer of myofibroblastic activation. Variable types of hepatocellular damage are apparently present in the nonlesional liver of

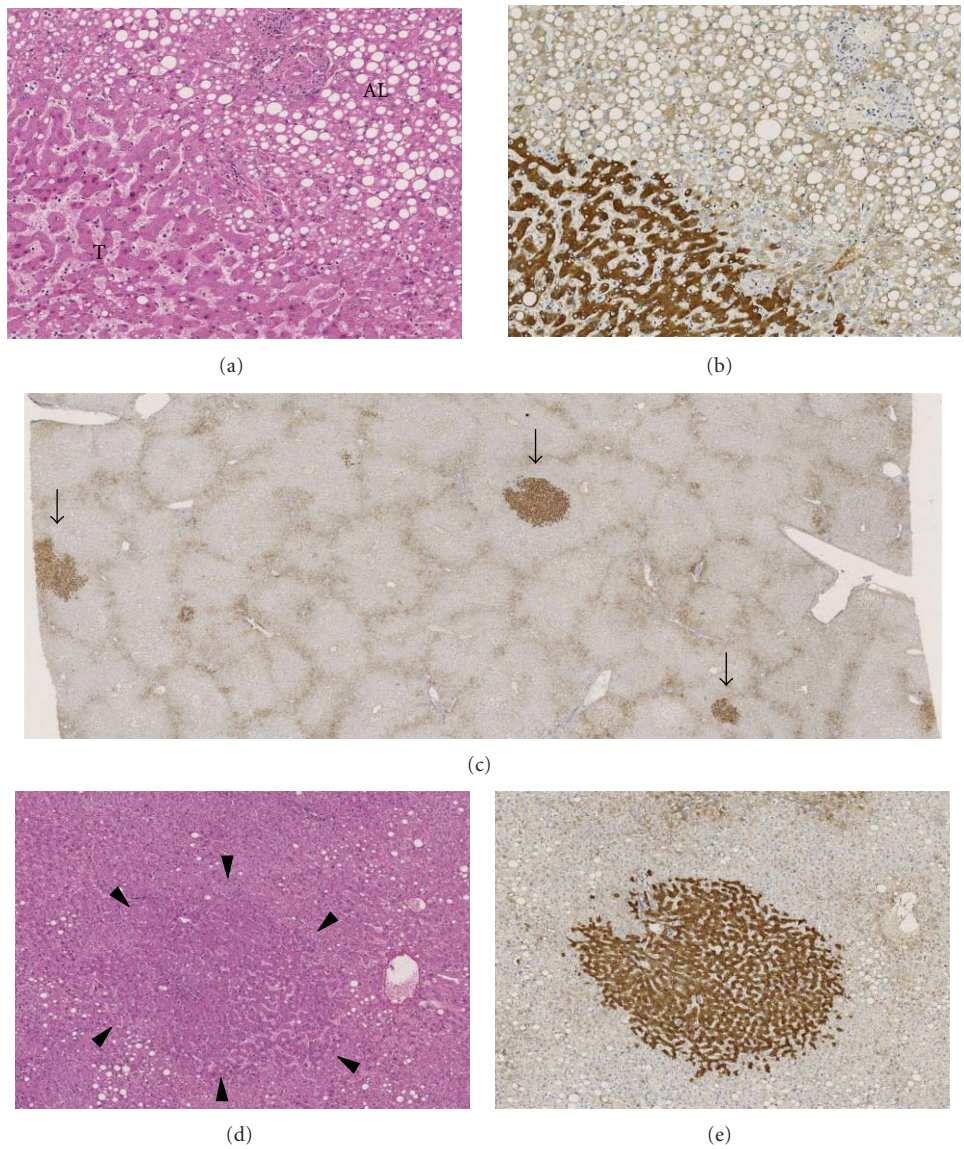
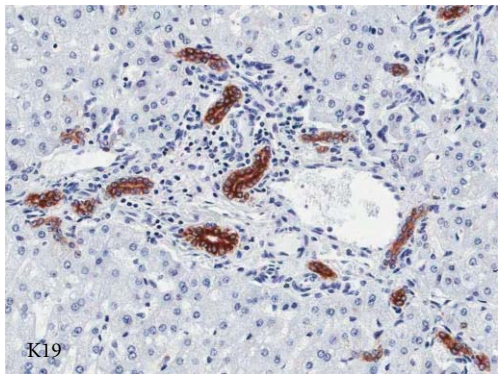


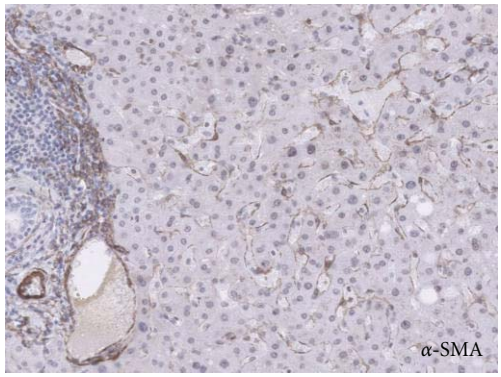
Figure 3: CRP expression in the transitional area and minute CRP-positive foci in a distant sample. (a) The transitional zone of an adenoma without steatosis (T) and steatotic adjacent liver (AL) in hematoxylin-eosin (HE) staining. (b) The same area as (a) in CRP immunostaining showing diffuse increase of CRP in the tumor part. (c) CRP immunostaining of a nontumorous liver sample distant from the tumor showing 3-minute CRP foci (arrows). The preserved architecture of the hexagonal liver lobules is highlighted by the vague expression of CRP which outlines the peripheral boundaries of the lobules. (d) Detail of a minute CRP-positive focus in HE showing an area in the lobule with slightly dilated sinusoids, more eosinophilic hepatocytes, and absence of steatosis which is present outside the contours of this focus (arrowheads). (e) The CRP expression of the focus described in (d).

IHCA which would require replenishment of cellular loss. The presence of ductular reaction in nearly all samples reflects the regenerative activity. Ductular reaction has been described in fatty liver disease [24], but in general, it reflects a reparative activity that includes several progenitor cell niches [14].

Apart from the degenerative changes, the findings of CRP-positive foci outside the tumor and within liver tissue with preserved lobular architecture are most intriguing. It is tempting to speculate that those foci may represent minute HCA, particularly because most of those foci were found in cases with multiple adenomas. Our findings largely confirm the results of Bioulac-Sage et al. who also found additional CRP-positive micronodules in multiple IHCA, measuring between 2 to 10 mm, and containing features of IHCA [25]. These micronodules are mostly slightly larger than the CRP foci in the present study which are mostly smaller than 2 mm and in the majority of cases were found in random samples. Due to its subtlety, these foci are easily overlooked, first during gross examination of the resected



(a)



(b)

Figure 4: Ductular reaction and activated myofibroblasts in the nontumorous liver. (a) Ductular reaction in a portal tract of a distant nontumorous liver sample highlighted by K19 immunostaining. (b) Presence of α-SMA-positive myofibroblasts in a distant nontumorous liver sample, most obvious in dilated sinusoids.

specimen and secondly in routine HE staining. This may probably lead to the reported absence of these foci in many other cases of multiple IHCA. To avoid this sampling error it is recommendable to investigate the nonlesional liver tissue more robustly, for example, to include more sampling and application of additional CRP staining. If positive foci are found, it may indicate the presence of minute foci of HCA in the remnant liver which may have consequences for the followup management. The long-term behavior of small HCA foci and under specific circumstances such as pregnancy is not fully established. A recent study on the management of HCA during pregnancy has shown that discouragement of pregnancy in certain cases is no longer necessary because close monitoring of patients with small adenomas seems to offer adequate surveillance [26]. Whether this applies to multiple adenomas with multiple CRP positive foci is yet unclear.

In conclusion, from the architectural point of view, the nonlesional liver part of IHCA may be considered normal. However, the CRP-positive foci indicate that in cases with multiple adenomas, minute foci of adenomas may be present, also in the remnant liver. The presence of vascular abnormalities beyond the tumor and beyond the CRP foci needs further study, especially due to the similarities with the changes in the HCA. The high incidence of steatosis does not only confirm the hepatic manifestation of obesity in this group of patients. It provides another evidence that the normal liver in IHCA does contain abnormalities and in selected cases should probably be considered as a diseased liver.

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Review Article

Overview of Hepatocellular Adenoma in Japan

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Hepatocellular adenoma (HCA) is generally a benign hepatocellular tumor arising in a nonfibrotic/cirrhotic liver, and recently four major subgroups were identified based on genotype and phenotype classification from Europe. HCA is rare in Asian countries including Japan, and there have been few studies regarding the subgroups of HCA in Japan. We surveyed subgroups of HCA in 13 patients (7 women) in Japan, based on the phenotypic classification. As results, we identified 2 hepatocyte nuclear factor (HNF) 1α-inactivated HCAs (15%), two β-catenin-activated HCAs (15%), 5 inflammatory HCAs (39%), and 4 unclassified HCAs (29%). The use of oral contraceptives was found only in 2 unclassified HCAs (29%). Rather low percentage of female patients and use of oral contraceptives appear to be common clinicopathological features in Japan and also East Asian countries. Furthermore, a group of possible inflammatory HCAs characterized by strong immunoreactivity for serum amyloid A (SAA) was found in patients with alcoholic cirrhosis. The inflammatory HCA/SAA-positive hepatocellular neoplasm in alcoholic cirrhosis may be a new entity of HCA, which may have potential of malignant transformation. Further studies are needed to clarify genetic changes, monoclonality, and pathogenesis of this new type of hepatocellular neoplasm.

1. Introduction

Hepatocellular adenoma (HCA) is benign monoclonal tumors occurring essentially in young women taking oral contraceptives (OCs) [1]. Eighty-five percent of cases occur in young women; HCA is rare in children, men, and the elderly [1–5]. The incidence of HCA is about 3-4 per 100,000 populations in Europe and North America [1], but lower in Asian countries, including Japan. HCA represents a heterogeneous entity, recently subclassified into several groups according to genotype and phenotype [2–6]. Recurrent mutations were identified in the HNF1A (hepatocyte nuclear factor 1 A) gene encoding the HNF1α, in the CTNNB1 (catenin (cadherin-associated protein), β1) gene coding for β-catenin, and recently in the interleukin 6-signal transducer gene encoding the signaling coreceptor gp130 [7]. In inflammatory HCA, about 60% of patients have small in-frame deletions in gp130 [7] and additional 12% carried activating STAT3 mutations [8]. Genotyping allowed the identification of three subtypes: HNF1α-inactivated HCA (35%–50% of cases), β-catenin-activated HCA (15%–18% of cases), and inflammatory HCA (40%–55% of cases), which could be identified by immunohistochemistry on paraffin-embedded materials [2–6]. Less than 10% of HCAs did not express any of these phenotypic markers (unclassified HCA). Few reports have examined the clinicopathological features and subtypes of HCA in Asian countries, including Japan [9–11]. Therefore, we have started to survey patients with HCAs in Japan and compared clinicopathological features with previous reports from other area.

2. Phenotypic Classification of HCA Subgroups

Immunohistochemical markers used for the phenotypic classification include liver fatty acid binding protein (LFABP), glutamine synthetase (GS), β-catenin, and serum amyloid A (SAA). LFABP-negative, GS-positive and/or nuclear β-catenin-positive, and SAA- positive HCAs are regarded as HNF1α-inactivated, β-catenin-activated, and inflammatory HCAs, respectively [2–6]. The remaining HCAs without specific markers are regarded as unclassified HCAs.

Table 1: Clinical and pathological findings of hepatocellular adenoma.

	HNF1α-inactivated ($n = 2$)	β-catenin-activated ($n = 2$)	Inflammatory HCA ($n = 5$)	Unclassified ($n = 4$)
Patients				
Age; mean ± SD (range)	40.5 ± 4.9 (37–44)	28.5 ± 3.5 (26–31)	40.2 ± 22.8 (15–68)	33.5 ± 14.1 (16–45)
Sex (M/F)	0/2	1/1	4/1	1/3
Oral contraceptives	0	0	0	2
Alcohol >40 g/day	0	0	2	0
BMI >25	0	0	2	0
Other clinical background	Portal hypoplasia 1	Hyperlipidemia 1	Diabetes 2	FAP 1
Background liver				
Steatosis	0	1	3	2
Fibrosis (F1/2/3/4)	0	0	3 (2/1/0/0)	2 (1/1/0/0)
Tumor				
Tumor size; mean ± SD (range)	4.0 ± 1.4 (3–5 cm)	17.5 ± 2.1* (16–19 cm)	3.9 ± 2.6 (1.6–9 cm)	4.4 ± 2.6 (1.5–7 cm)
Unique/multiple	1/1	2/0	3/2	1/3
Steatosis	1	1	2	1
Association of HCC	0	0	0	1

HNF1α: hepatocyte nuclear factor 1α; HCA: hepatocellular adenoma; M: male; F: female; BMI: body mass index; FAP: familial adenomatous polyposis; HCC: hepatocellular carcinoma; *$P < 0.05$, compared to other groups.

3. Clinical and Pathological Features of HCAs in Japan

Table 1 summarizes the clinical and pathological features of each subtype based on the new phenotypic classification [10]. We retrieved 13 HCA cases from our pathological files from 1997 to 2011 and surveyed a phenotypic classification of HCAs. In addition, we found 7 patients with alcoholic cirrhosis and hepatocellular neoplasms showing the immunoreactivity for SAA as same as inflammatory HCAs in same series. Since HCA usually arises in nonfibrotic/cirrhotic livers, "HCA in cirrhotic liver" appears to conflict with a general concept. Therefore, we tentatively called these possible HCAs as "SAA-positive hepatocellular neoplasms" [12] and did not include them in the present study. We will discuss on this possibly new type of HCA, as described below.

Patients with HCAs included 6 men and 7 women and their age ranged 15–68 yrs (41.0 ± 13.9 yrs). We identified 2 HNF1α-inactivated HCAs (2 women), two β-catenin-activated HCAs (one woman), 5 inflammatory HCAs (one woman), and 4 unclassified HCAs (3 women). The use of OCs was found in only 2 unclassified HCAs (29% of women). Figure 1 shows the representative histology and immunohistochemical findings of inflammatory HCAs. Two inflammatory HCA patients (one woman) were obese (BMI >25) and 2 inflammatory HCAs (all men) had diabetes. Mild hepatic fibrosis liver was seen in the background in 3 inflammatory HCAs (60%) and 2 unclassified HCAs (50%). Steatosis was seen in the background liver in one β-catenin-activated HCA, 4 inflammatory HCAs (36%), and 2 unclassified HCAs (50%). Six patients (46%) had multiple HCAs; one HNF1α-inactivated HCA, 2 inflammatory HCA, and 3 unclassified HCAs. All multiple HCAs were of the same type in each case. The tumor was significantly larger in β-catenin-activated HCAs than in other subtypes. The association of hepatocellular carcinoma was seen in only one case of unclassified HCA. A definite diagnosis of HCC was made based on cytological abnormalities, a loss of reticulin fiber in a focal area in this HCA. In addition, this area showed the immunoreactivity for glypican-3, which is a marker of HCC.

4. Features of HCAs in Japan: A Comparison with Previous Reports

Table 2 summarizes a comparison of HCAs reported from Japan [9, 10], Europe [3], and China [11]. Our study revealed different clinical and pathological features of HCAs in Japan from those in Europe and North America [1–5, 9, 10]. The features of HCAs in Japan can be listed as follows: (1) a half of HCAs occur in men; (2) a use of OCs is infrequent; (3) a rate of HNF1α-inactivated HCAs is rather low.

Gender and OCs. Compared with previous studies reported from Western countries [1–5, 13], in which more than 90% of patients with HCAs were women, lower percentage of female patients appears to be a feature of HCAs in Japan [9, 10]. A half of HCAs occur in men in our study. This result agrees with a previous study from Japan, in which about 60% of patients with HCAs were women [9]. Interestingly, the percentage of female patients was further less in the study from China [11]. The use of OCs is infrequent in HCAs in Japan in our study and a previous report [9], whereas the use of OCs was noted in about 80–90% of female patients with HCAs in Europe [3, 13]. The use of OC was also infrequent in China [9]. A lower rate of obesity might be a reason for the difference. The high frequency of men seems to be related to rather low rate of HNF1α-inactivated type. In addition,

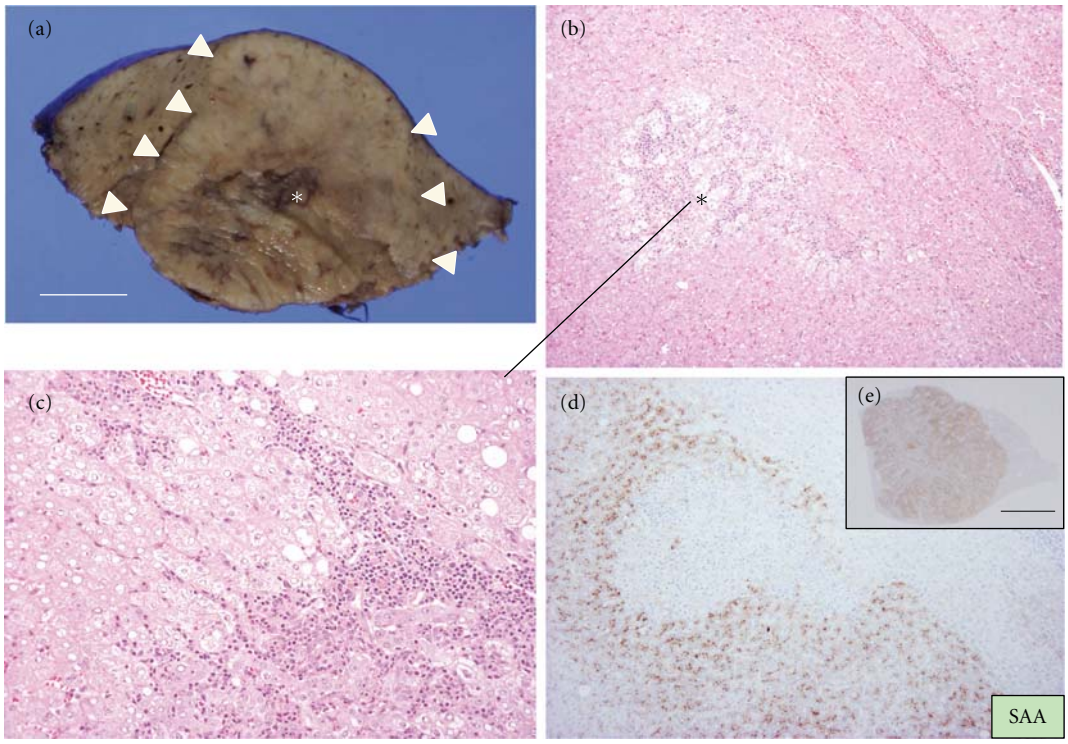

Figure 1: Inflammatory hepatocellular adenoma (HCA). (a) Cut surface shows whitish nodular lesion sized 3 cm in diameter (arrowheads). There was no capsule and the border between nodule and background liver is unclear. Congestion or bleeding is observed inside the nodule (asterisk). Bar = 1 cm. (b) Border of HCA and background liver. Ductular reaction is focally seen in HCA (asterisk). HE, ×100. (c) Ductular reaction with lymphoid cells infiltration in HCA. HE, ×200. (d) Strong expression of serum amyloid A component (SAA) in HCA in contrast to the background liver. Immunostaining for SAA and hematoxylin. ×100. (e) bar = 1 cm.

Table 2: Hepatocellular adenoma in Japan: a comparison with previous reports.

	Sasaki et al., 2011 [10]* (n = 13)	Konishi et al., 1995 [9] (n = 58)	Lin et al., 2011 [11] (n = 191)	Bioulac-Sage et al., 2009 [3] (n = 128)
Age; mean (range)	41 (15–68)	34	39 (5–79)	41 (21–66)
Female	54%	62%	38%	91%
Oral contraceptives	29%	8%	4.2%	78%
Alcohol >40 g/day	15%	nd	nd	12%
BMI >25	15%	nd	nd	33%
Tumor size; mean (range) (cm)	5.3 (1.5–19)	11.3	8.3 (2–31)	7 (1–18)
Multiple	46%	16%	6%	39%
Association of HCC	8%	2%	6%	4%
Subtypes	HNF1α-inactivated: 15% β-catenin-activated: 15 IHCA: 39% Unclassified: 31%	nd	nd	HNF1α-inactivated: 38% β-catenin-activated: 6% IHCA: 52% Unclassified: 5%

HNF1α: hepatocyte nuclear factor 1α; IHCA: inflammatory hepatocellular adenoma; BMI: body mass index; *with modification.

HCC, not HCA, is generally suspected for hepatic nodules arising in men. In fact, partial hepatectomy was performed in male patients in our survey, since HCC was suspected. Same nodules might be diagnosed in HCA in women and would be followed up without resection. The previous study in Japan reported a higher rate of male patients with HCAs associated with predisposing factors, such as glycogen storage disease and anabolic hormone treatment for aplastic anemia [9]. Such predisposing factors were not noted in the patients with HCAs in our recent study [10]. Taken together, lower percentage of female patients and use of OCs may be common characteristics of patients with HCAs in East Asia.

Tumor Size and Multiplicity. Tumor size ranged 1–31 cm and mean of tumor size was 5.3 cm, 11.3 cm, 8.3 cm, and 7 cm in each study (Table 2) [3, 9–11]. A mean tumor size is rather big in the previous study from Japan reported in 1995 [9]. Small HCAs may be able to be detected earlier because of recent progress of imaging modalities. Multiple tumors were detected in 46% and 39% of patients in the present study and study from France [3], respectively. In contrast, only 6% was diagnosed as multiple lesions in the study from China [11]. The reason for this difference is unclear.

Association of HCC. The malignant transformation of HCA to HCC has been described in previous studies [2, 3, 14–16]. Association of HCC is 8% in our present study and it ranged 2–6% in other studies [3, 9, 11]. The risk of HCC is linked to β-catenin mutation [2, 3, 14]. Larger size and male gender are also related to the association of HCC [11, 14]. The patient with HCA with malignant in our present study was a man having unclassified HCA, 5 cm in size. In addition, coexistence of chronic hepatitis B virus infection was suggested to be a risk factor for malignant transformation in the report from China [11].

Subgroups. We have reported for the first time a proportion of subgroups of HCAs in Japan and Asian countries, to our knowledge [10]. In our present study, a percentage of HNF1α-inactivated HCA was lower than the study from France [2, 3]. Recently, Fukushima et al. surveyed subgroups of 14 Japanese patients with HCAs and reported the proportion was as follows: HNF1α-inactivated, 18%; HNF1α-inactivated and β-catenin—activated 18%, β-catenin—activated, 12%; inflammatory HCA, 29%; unclassified, 24% [17]. A lower rate of HNF1α-inactivated HCA may be a feature of HCAs in Japan. Since HCA associated with mutant HNF1A, a major type of HNF1α-inactivated HCA, occurs almost exclusively in women [2–6], higher rate of male patients may be a reason for a lower rate in this subgroup. Inflammatory HCA is a major subgroup in this study, consistent with recent studies in Europe [3, 13]. Therefore, it is conceivable that inflammatory HCA may be a common major subgroup of HCAs, irrespective of race and region. It is not clear why the rate of unclassified HCA was rather high in our survey. These unclassified HCAs did not show the immunoreactivity for CRP, another marker of inflammatory HCA. Furthermore, these unclassified HCA did not show histological characteristics of inflammatory HCA, such as ductular reaction and inflammatory cell infiltration. Mild steatosis was seen in one unclassified HCA.

5. Serum Amyloid A-(SAA) Positive Hepatocellular Neoplasm in Alcoholic Cirrhosis: A Possible New Subtype of HCA

Our previous study highlights a group of hepatocellular nodules in alcoholic cirrhosis which conflicts with the general concept of "HCAs" [10]. In fact, the liver lesion with solid mass arising in fibrotic/cirrhotic background is not supposed to be an HCA according to the diagnostic algorithm proposed in WHO classification 2010 [2]. Histologically, these nodules showed features of inflammatory HCAs, such as sinusoidal dilatation, inflammatory cell infiltration, and ductular reaction (Figure 2). Immunohistochemically, these nodules showed strong immunoreactivity for SAA as same as inflammatory HCAs (Figure 2). Immunostaining for GS showed weak and diffuse pattern in these nodules, which was different from focal nodular hyperplasia (FNH) showing map-like pattern. Taken together, we named this unique type of possible HCA as "SAA-positive hepatocellular neoplasm" [12] and have been doing further studies. SAA-positive hepatocellular neoplasms included some hypervascular hepatic nodules showing similar imaging findings to HCC; the so-called FNH-like nodules, or drinker's nodules, occur in severe alcoholic fibrosis or cirrhosis [18–20]. In our survey, six patients with SAA-positive hepatocellular neoplasms were found in 10 patients with the so-called FNH-like nodules and alcoholic cirrhosis. Further studies including molecular analysis are necessary to establish this new type of nodular lesions.

It is well known that alcohol is a risk factor of HCC. In addition, metabolic syndrome is a newly identified risk factor in chronic liver disease and HCC [21–23]. Alcohol, obesity, and fatty liver diseases are frequent in inflammatory HCAs [2, 3]. So, HCAs may be precursor lesions of HCC in metabolic syndrome. Similarly, SAA-positive hepatocellular neoplasms may be precursor lesions of HCC. SAA-positive hepatocellular neoplasms showed different features from HCC in the immunoreactivity for GS and glypican-3 [12]. Most SAA-positive hepatocellular neoplasms were negative for glypican-3 in our recent study [12]. Therefore, glypican-3 is useful to differentiate SAA-positive hepatocellular neoplasms from HCCs. HSP70 is also reportedly useful to differentiate benign and malignant tumors [24], so this marker may be useful. The CD34-positive capillarization of sinusoid was seen in both SAA-positive hepatocellular neoplasms and HCCs in our preliminary study, so CD34 may not be a definite marker for differential diagnosis. However, it should be noted that histological diagnosis between HCA and well-differentiated HCC may be difficult in some cases. Interestingly, Farges et al. have recently reported changing trends in malignant transformation of HCA [14]. Prevalence of malignancy within HCA is 10 times more frequent in men than in women in their study [14], and they propose that management of HCA should primarily be based on gender. Whereas oral contraception is a classical cause of HCA in women but a marginal cause of HCC, the metabolic syndrome appears as an emerging condition associated with malignant transformation of HCA in men and is the likely predisposing condition for HCC in this setting [14]. Taken together, clinical and pathological features SAA-positive hepatocellular neoplasm, a possible inflammatory HCA, should be examined in larger series to confirm the significance of unique lesion.

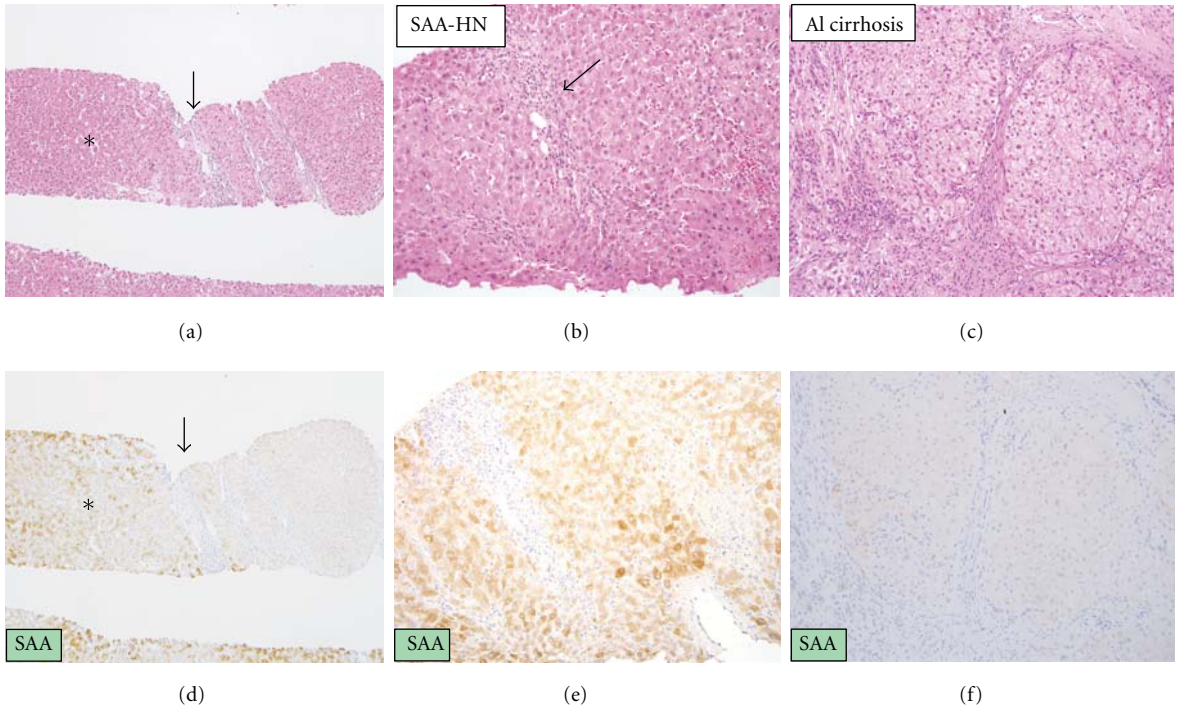

Figure 2: Serum amyloid A-positive hepatocellular neoplasm associated with alcoholic cirrhosis. (a) The boundary between serum amyloid A-positive hepatocellular neoplasm (SAA-HN) (asterisk) and nontumor tissues. Arrow indicates the boundary. ×40. (d) Serum amyloid A is expressed in the tumor, whereas it is negative in the background liver. Immunostaining for serum amyloid A, ×40. (b) Serum amyloid A-positive hepatocellular neoplasm (SAA-HN). Inflammatory cells infiltration, focal sinusoidal dilatation and ductular reaction are seen in the tumor. HE, ×200. (e) The cells in the nodule show extensive granular immunoreactivity for serum amyloid A. Immunostaining for serum amyloid A, ×200. (c) Background liver shows established micronodular cirrhosis consistent with alcoholic cirrhosis. HE, ×200. (f) The background liver shows negative immunoreactivity for serum amyloid A. Immunostaining for serum amyloid A, ×200.

6. Concluding Remarks

Clinical and pathological features of HCAs appear to be different in East Asia including Japan and Western countries. Lower percentage of female patients and use of OCs may be common characteristics of HCAs in East Asia including Japan. Our recent studies highlight a characteristic group of hepatocellular neoplasms arising in alcoholic cirrhosis, which share features with inflammatory HCAs. These SAA-positive hepatocellular neoplasms may be a new type of hepatocellular tumors having a potential of malignant transformation in alcoholic patients.

Conflict of Interests

The authors declare that there is no conflict of interests.

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