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Will the pathomolecular classification of hepatocellular adenomas improve their clinical management?

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Hepatocellular adenomas (HCA) are rare benign tumors. Women taking oral contraceptives (OC) represent the most classical circumstance of HCA appearance. In recent years, clinical diagnosis of focal nodular hyperplasia (FNH) vs HCA has significantly improved due to the wide use of contrast agent ultrasonography and magnetic resonance imaging (MRI). However, there are circumstances where the differential diagnosis remains difficult and consequently require specimen resection. In addition, the diagnosis of benign hepatocellular nodules has greatly benefited from the contribution of molecular biology [1–3] allowing for the differential diagnosis between FNH and HCA and the identification of HCA subtypes. To date three major subtypes have been identified: *HNF1A* mutated HCA (H-HCA), β-catenin mutated HCA (β-HCA), and inflammatory HCA (IHCA). IHCA can be also β-catenin mutated (β-IHCA). Less than 10% of HCA remain unclassified. A phenotypic classification of those benign tumors using immunohistochemistry has been derived from the above-mentioned molecular characterization. Liver fatty acid binding protein (LFABP), serum amyloid A (SAA)/C reactive protein (CRP), glutamine synthase (GS), and β-catenin immunohistochemical analyses performed on surgical specimens discriminate FNH from HCA and identify the different HCA subtypes (Table 1) [4–8]. The advantages of immunohistochemistry-based approaches are (i) simplicity (can be performed on formalin fixed, paraffin-embedded tissue), (ii) reproducibility and (iii) easiness to analyze (in most cases). Briefly, the use of molecular markers and its pathological counterparts has allowed (i) to clearly separate FNH from HCA by introducing the previously called telangiectatic FNH [9] in the subgroup of IHCA, (ii) to identify different clinical, biological, radiological, and pathological HCA subtypes and most importantly (iii) to identify HCA at risk to malignant transformation into hepatocellular carcinoma (HCC) [10]. One of the most interesting discoveries resulting from these analyses was the highest percentage of overweight patients with IHCA (β-catenin mutated or not) compared to other subtypes and the presence of an inflammatory syndrome in IHCA [11].

In this issue [12] Van Aalten and co-workers report their experience using the HCA classification described above [4]. They confirm that immunohistochemistry markers are useful in typing HCA in more than 90% of cases tested and that this classification, including the identification of β-catenin positive HCA may have important implications in the decision process for surveillance or treatment. They analyze 71 lesions of which 14 were identified as FNH and 57 as HCA. Van Aalten and colleagues also confirm that the map-like pattern of GS staining was very useful to characterize FNH. The population studied in terms of age, gender, clinical manifestations including bleeding, number, and size of nodules was unremarkable. Of note, none of the specific HCA etiology (i.e. glycogenosis) was present. Among the HCA identified in this study, 19% (11 cases) were LFABP negative, 63% (36 cases) were IHCA expressing SAA and CRP (4 expressing GS and 3 out 4 aberrant β-catenin), 7% (4 cases) were β-catenin positive, and 11% (6 cases) remained unclassified. The numbers reported are of the same magnitude than those obtained in France on a large population using molecular and/or immunohistochemistry [11]. The sample series analyzed in the Van Aalten study was, however, too small to draw any valid conclusion concerning the influence of subtype on hemorrhagic risk, size, body mass index, follow-up for unresected HCA, malignant transformation even in β-catenin mutated HCA. Of interest in their paper was the presence of two different subtypes in the same patient. Unclassified HCA were associated with a β-catenin mutated HCA in one case and with IHCA in another case. These data could suggest, as the authors mentioned, that unclassified HCA may be acquired with time with another phenotype, but this needs to be confirmed in a larger series and with molecular tools.

Will the pathomolecular classification of HCA improve their management? (Fig. 1) Will it change our policy based on size [11,13]? At the present time, the policy is to resect tumors ⩾5 cm to avoid complications (hemorrhage and HCC). To be able to answer this question, it is necessary to classify the tumors

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Abbreviations: HCA, hepatocellular adenoma; OC, oral contraceptives; FNH, focal nodular hyperplasia; MRI, magnetic resonance imaging; H-HCA, HNF1A mutated HCA; β-HCA, β-catenin mutated HCA; IHCA, inflammatory HCA; LFABP, liver fatty acid binding protein; SAA, serum amyloid A; CRP, C reactive protein; GS, glutamine synthase; HCC, hepatocellular carcinoma.

Table 1. HCA subtypes: pathological and immunohistochemical features for diagnosis.

Stains/IHC*		HNF1α	β-cat	IHCA	IHCA/β-cat	Unclassified
H&E[1]	HCA	diffuse steatosis	rosette formation some cytological atypia	inflammatory infiltrate sinusoidal dilatation thick arteries ductular reaction	idem IHCA + β-cat	no specificity
	NT			frequent steatosis (obesity)	frequent steatosis (obesity)	
LFABP*	HCA	-	+	+	+	+
	NT	+	+	+	+	+
SAA/CRP*	HCA	-	-	+	+	-
	NT	-	-	-	-	-
β-cat*	HCA	N	+[2]	N	+[2]	N
	NT	N	N	N	N	N
GS*	HCA	-[3]	+[4]	-[3]	+[4]	-[3]
	NT	5	5	5	5	5

HCA: hepatocellular adenoma; IHC*: immunohistochemistry; NT: non tumoral liver; H&E: hematoxylin eosin (1: major findings not always observed); LFABP: liver fatty acid binding protein (+: normal hepatocytes staining); SAA: serum amyloid A/CRP: C reactive protein (+: diffuse hepatocytes staining); β-cat: β-catenin (N: normal membranous staining; 2: aberrant cytoplasmic/nuclear staining can be diffuse, focal or limited to few hepatocytes); GS: glutamine synthase (3: GS staining is usually absent, occasionally some hepatocytes can be stained at the periphery of the nodule as well as around veins; 4: GS staining is usually strong and diffuse; when the staining is heterogeneous (intensity and location) the interpretation is difficult; in this case β-catenin mutation should rely on β-catenin staining or molecular analysis; 5: normal GS staining limited to few centrolobular hepatocytes).

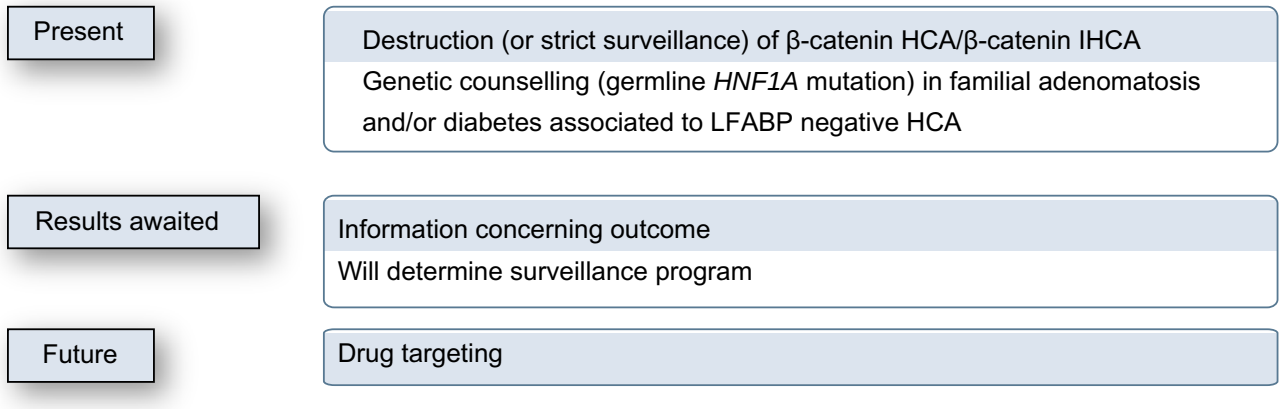

Fig. 1. Reasons for the identification of HCA subtypes (<5 cm).

prior to surgery. The radiological diagnosis of HCA on MRI is possible in H-HCA and IHCA at least in typical cases [14]. However, more data are necessary in order to estimate the true diagnostic accuracy. One of the major limitations that remains is in the identification of β-catenin mutated HCA. Indeed, it is important to identify the β-catenin mutation because it is likely to be a marker of premalignant HCC transformation even before traditional pathological criteria are present (i.e. some cytological abnormalities, rosette formation...). β-Catenin mutations occur most frequently in specific conditions, such as glycogenosis, male hormone administration (Danazol), male patient, etc. Unfortunately it occurs also in the absence of etiological criteria. Unless sampling all HCA (except H-HCA), it appears more reasonable to follow the growth of HCA after stopping OC. HCC transformation might be unlikely if the size of the HCA decreases whereas any size increase might become suspicious. In the 4 cm range or over if surgery is not planned, it appears reasonable to recommend a biopsy (allowing for HCA subtyping) and to follow the patient in the absence of the β-catenin mutation.

The role of the biopsy has not yet been objectively evaluated. Preliminary data from a multicentric French study [15] reveal that the use of the same immunohistochemistry markers is promising, particularly in the two main categories of H-HCA and IHCA, as well as to differentiate HCA from FNH. Interpretation of immunohistochemical results may, however, even on surgical specimen be problematic [7]. Indeed the identification of β-catenin mutated HCA remains the main issue, with variable difficulties, particularly on biopsy, but sometimes also on surgical specimens. The easiest situation corresponds to a strong/diffuse GS staining associated with the presence of β-catenin labeled

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nuclei (a few or numerous) that is constantly associated with an activating β-catenin mutation. However, strong GS staining without nuclear β-catenin or heterogeneous distribution of GS in the absence of β-catenin labeled nuclei remains difficult to interpret [7]. This was confirmed by Van Aalten and colleagues. In our experience, GS and β-catenin immunohistochemical staining is not sensitive enough to detect all β-catenin mutations in HCA (85% sensitivity [1]). Therefore, it is particularly important that pathologists collect frozen material for each surgical specimen of these tumors to search for β-catenin mutations or small deletions in LFABP positive and IHCA.

In addition to the identification of β-catenin mutations, the importance of HCA subtyping can be seen in several ways. First, we know that specific genetic factors are associated with the development of HCA [16–18]. Second, for patients with LFABP negative HCA and familial adenomatosis or maturity diabetes of the young (MODY), we propose a genetic counseling and a germline *HNF1A* mutation screening.

Other questions remain to be elucidated: is the risk of bleeding, the evolution of nodules (growth, regression) after stopping OC (whether unique, or remaining after resection of the largest one) subtype dependent? Is the distribution of subtypes different according to gender, age (infants, adolescents, menopausal women), in women never exposed to OC, and in patients subject to vascular disorders? Is the risk of bleeding subtype dependent in women who want to become pregnant?

The most appealing usefulness of HCA subtyping will be to provide us with better tools to define patient treatment/surveillance guidelines once HCA are discovered. Since the first paper showing the relationship between OC and HCA was published in 1972, the overall picture of HCA has dramatically changed. Indeed today, estrogen concentrations have decreased, OC are often prescribed at a younger age, women seldom observe to refrain from smoking and obesity is becoming a major health problem. In the meantime smaller nodules are discovered more frequently, and the discovery of HCA in post-menopausal women is no longer an exception.

HCA remain rare tumors even in countries where OC are widely prescribed. Therefore, we make a pledge that in countries where HCA are observed, particularly in Europe, radiologists, surgeons, pathologists, and hepatologists from referral centers should combine their expertise with the expertise of molecular biologists in collaborative research programs to improve our understanding of the disease and to better adapt treatments. It is also important to mention that as patients are eager to access relevant information on the web, we must provide them with comprehensive, consensual, and validated data and in this line van Aalten and collaborators here provide the first international validation of the HCA pathomolecular classification.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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