Liver precancerous lesions and hepatocellular carcinoma: The histology report

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Abstract

The current ability to increase the survival of patients with hepatocellular carcinoma (HCC) relies upon the surveillance of cirrhotic patients. Surveillance allows HCC precursors (dysplastic nodules) and malignant tumors to be recognized at an earlier stage making cure possible. Radiology plays a major role in HCC diagnosis because HCC is characterized by neoarterial vascularisation with a typical imaging pattern. Current international guidelines have restricted the use of the liver biopsy to the characterization of hepatocellular nodules which remain diagnostically equivocal after imaging. Thus pathologists are today facing very challenging and often well differentiated lesions, leading to difficulties in distinguishing high grade dysplasia and well differentiated HCC. In this scenario novel concepts obtained through international consensus have been proposed with emphasis on HCC of small size (up to 2 cm) which includes 2 distinct types, the early and progressed HCC. In this paper we will report the main histopathological criteria of a biopsy which allow the differentiation of HCC precursors (dysplastic nodules) from well differentiated HCC with attention to the role and weight of both classical histopathological criteria and novel immunocytochemical markers. The second part of the paper is devoted to the histopathology report of HCC on surgical specimens including explanted livers and on the differential diagnosis between HCC and liver metastasis.

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1. Introduction

A variety of hepatocellular nodules (hyperplastic, benign, dysplastic and malignant) and secondary tumors can be detected in the liver. The occurrence of these lesions is substantially different according to the liver background (hepatitic/cirrhotic vs. non-hepatitic). In the hepatitic liver hepatocellular carcinoma (HCC) and nodular precursors such as regenerative (LRN)/dysplastic (low-grade LGDN and high grade HGDN) nodules related to hepatocarcinogenesis, far outnumber all the other lesions. Conversely in non-hepatitic liver, metastases are more common than primary benign (focal nodular hyperplasia and adenoma) and malignant (HCC and cholangiocarcinoma) neoplasms.

In this paper we will summarize the diagnostic issue of HCC in the hepatitic liver with particular attention to tumors of small size and to the differential diagnosis with dysplastic

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nODULES, AS DETECTED IN RESECTED SPECIMENS AND IN THE LIVER BIOPSY. WE WILL THEN FOCUS ON LIVER METASTASES THAT USUALLY TAKE PLACE IN THE SETTING OF NON-HEPATITIC LIVER, WITH EMPHASIS ON THE DISTINCTION BETWEEN PRIMARY AND SECONDARY TUMORS AND ON THE CLUES TO ADDRESS THE ISSUE OF THE ORIGIN OF THE METASTATIC DISEASE. FOCAL NODULAR HYPERPLASIA, ADENOMA, VASCULAR AND NON-EPITHELIAL TUMORS WILL NOT BE COVERED.

2. EPIDEMIOLOGY OF HCC

HCC is a global health problem, representing the third cause of death for cancer and the fifth most common cancer worldwide. In all areas of the world the incidence is higher in males than in females (M/F ratio ranging from 3.3 to 1.4). Liver cancer incidence has increased in Japan and many Western countries in the last decades. The incidence varies depending on the prevalence of the major causes, namely chronic liver disease due to HBV and HCV hepatitis or to other diseases. The most common risk and predisposing condition worldwide is chronic HBV infection (52.3% of all HCC) while HCV infection accounts for 20% of all HCC [1]. Whatever the underlying liver disease, cirrhosis is a major factor able to modulate HCC risk, the annual risk of developing HCC in cirrhosis being between 1% and 6%. However, at variance with HCV hepatitis, in HBV-related hepatitis 40% of patients with HCC do not have cirrhosis but the liver is seldom normal and may show changes due to regressed cirrhosis [1].

3. CLINICAL ASPECTS AND SURVEILLANCE FOR EARLY DETECTION OF SMALL HCC AND NODULAR PRECURSORS

In the absence of surveillance and early detection programs, HCC presents late in the course of the disease with the onset of symptoms. At this stage of the disease curative therapy can seldom be applied safely and when feasible is not effective. Progression of disease is usually rapid with unfavourable short-term prognosis. To be successful surveillance should detect HCC at a stage when cure is possible: once HCC is larger than 2 cm the frequency of cure decreases compared to smaller lesions. Lesions larger than 3 cm are even less likely to be cured [1].

HCC arising in cirrhosis is usually preceded by the appearance of non-malignant precancerous lesions such as LRN/LGDN and HGDN. Thus the liver of a cirrhotic patient may harbor either a single benign/dysplastic or malignant nodule or even both. The prevalence of malignancy among hepatocellular nodules in the hepatic/cirrhotic liver under surveillance for early HCC detection is largely dependent on the size of the lesion, as shown in Table 1. Indeed, most of <1 cm lesions are non-malignant whereas the large majority of lesions exceeding 2 cm are HCC, so that in the group of lesions greater than 2 cm a diagnosis of non-malignancy should arouse the suspicion of a diagnostic error.

As to the natural history of nodular HCC precursors, Table 1: Non-malignant vs HCC nodules detected in cirrhosis during surveillance according to size

<table>
<thead>
<tr>
<th>Reference</th>
<th>Total</th>
<th>NM-HN/HCC 10–20 mm</th>
<th>NM-HN/HCC &gt;20 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolondi et al. [2]</td>
<td>72</td>
<td>12/60</td>
<td>12/29</td>
</tr>
<tr>
<td>Forner et al. [3]</td>
<td>74</td>
<td>16/58</td>
<td>16/58</td>
</tr>
<tr>
<td>Sangiovanni et al. [4]</td>
<td>63</td>
<td>21/42</td>
<td>20/34</td>
</tr>
<tr>
<td>Leoni et al. [5]</td>
<td>75</td>
<td>20/55</td>
<td>16/27</td>
</tr>
</tbody>
</table>

NM-HN: non-malignant hepatocellular nodule; HCC: hepatocellular carcinoma.

Borzio et al. [6] followed 90 cirrhotics with histologically proven LRN, LGDN and HGDN and found that the presence of HGDN or extranodular large cell changes (LCC) were associated with an increased hazard risk for malignant transformation (2.4 and 3.1 respectively over a mean follow up of 33 months). LRN and LGDN were associated with a lower propensity for HCC development. In another study, Kobayashi et al. [7] followed 154 patients who had histologically proven LRN, LGDN, HGDN for a median period of 2.8 years. The hazard ratios of HGDN, LGDN and LRN for transformation to HCC were 16.8, 2.96 and 1.0 respectively. Thus only a minority of regenerative/dysplastic nodules converted to malignancy (mostly in the group of HGDN), doing so in a relatively short interval, while 40–60% stabilized and a few definitely disappeared during follow-up.

HCC is fed by a neoarterial supply which is of cardinal importance for its detection through image analysis. Because the typical radiologic features of HCC are very specific [enhancement in arterial phase followed by washout in portal venous phase on cross-sectional imaging (CT or MRI or contrast-enhanced ultrasound)] biopsy is only required when the radiologic appearance is not typical. Indeed, current international guidelines indicate that nodules greater than 2 cm arising in cirrhotic patients, with typical features for HCC at one dynamic technique, do not require biopsy confirmation. However, if lesions reveal atypical features, which occurs in 10–15% of the cases [2,3], biopsy is required. Importantly, for nodules between 1 and 2 cm, two concordant radiological tests are needed for a non-invasive diagnosis of HCC, otherwise biopsy is recommended and this occurs in about 60–70% of the cases [2]. Thus, most of small incident nodules in cirrhosis, suspicious but not conclusive for HCC, are currently biopsied. In these cases HCC is mostly well differentiated and hypovascular (due to incomplete vascularization) so that the differential diagnosis with dysplastic nodules may be very challenging and only feasible on biopsy material. Biopsy provides both architectural and cytologic information which, in this specific setting, are superior to cytology, as has been recently acknowledged [8]. Some small nodules show up radiologically as “nodule in nodule”. In these cases the parent outer nodule may be dysplastic or well differentiated HCC while the smaller, inner subnodule is invariably malignant and less differentiated. Therefore a “nodule in nodule” lesion is always highly suspicious for malignancy.
4. Pathology

In the clinical practice pathologists face two main issues:
1. the spectrum and differential diagnosis of small (≤2 cm) hepatocellular nodules;
2. the sampling and the pathology report of HCC after resection/transplantation.

In the following paragraphs we will consider these 2 issues separately.

4.1. The spectrum and differential diagnosis of small (≤2 cm) hepatocellular nodules

4.1.1. Nomenclature

An international consensus [8] has recently been obtained on the classification of small (≤2 cm) hepatocellular nodules and their differential diagnosis as shown in Table 2.

4.1.2. Basic histopathological features

Non-malignant and dysplastic hepatocellular nodules are mostly less than 2 cm in diameter and usually between 0.5 and 1.5 cm. Imaging features are variable but rarely diagnostic, thus they are usually biopsied whenever showing, during follow up, size enlargement or changes in the imaging pattern. Grossly these nodules differ from the surrounding liver parenchyma with regard to size, color, texture and degree of bulging of the cut surface. Histological examination permits, in most cases, a careful distinction between low and high grade dysplastic nodules particularly on resected samples. Like the adjacent cirrhotic nodules dysplastic nodules are peripherically rimmed by fibrous septa forming a capsule. Histologically a common feature is the presence of portal tracts which are diagnostically helpful particularly in the liver biopsy and concur, together with more specific histological features, to ascertain whether a nodule has been correctly sampled. A ductular reaction (DR) is commonly seen in the isolated, intranodular portal tracts and in the outer capsule as well, either using H&E and the CK7/19 immunostaining as a surrogate marker for the DR [9]. It is recognized that a nodule well-decorated by a DR is not malignant because infiltrating HCC is rimmed by desmoplasia pushing apart or destroying the pre-existing DR. This is an important histological variable (level 3 of diagnostic strength).

Low grade dysplastic nodule (LGDN, Fig. 1): This category also includes so-called regenerative nodules (LRN) because morphological criteria to distinguish between LGDN and merely regenerative nodules have proved unreliable and unreproducible [8]. Usually this category features a nodule showing mild increase in cell density with a monotonous pattern and/or clonal changes (clear cells, eosinophilic cells, etc.). As compared to surrounding nodules, LGDN may show bland cytologic but not frank architectural atypia. LCC (large cell changes/large cell dysplasia) is frequently seen inside and outside the nodule as microscopic (<1 mm) dysplastic foci. Pathologists usually identify LGDN as non malignant on both liver biopsy and surgical specimens.

High grade dysplastic nodule (HGDN, Fig. 2) always shows a certain degree of cytological and architectural atypia but insufficient for a diagnosis of malignancy; as compared to adjacent cirrhotic nodules a common feature is the increased cell density (sometimes up to two times the surrounding non tumoral liver), often with an irregular trabecular pattern. Foci of SCC (small cell changes/small cell dysplasia) are frequently seen inside the nodule but LCC foci can also be detected. Unpaired non-triadal arteries occurring outside the portal tracts may also be seen, albeit not in a great number. Most pathologists will consider a well differentiated HCC in the differential diagnosis. Importantly a subnodule growing within a high grade dysplastic nodule is likely a well differentiated HCC.

Small and early HCC (HCC of vaguely nodular type or HCC with indistinct margins, Fig. 3). This lesion is characterized by the small size (≤2 cm), the deceptively replacing and not destructive pattern of growth, the incomplete or absent fibrous capsule at the tumor-non tumor boundaries, the little cellular and structural atypia [i.e. increased cell density (often more than twice the surroundings) and N/C ratio, irregularly thin trabeculae, some acinar/pseudoglandular structures (i.e. a very well differentiated histology)], the presence of scattered intratumoral portal tracts, the variable number of unpaired arteries and the frequent (40%) steatosis. All these atypical features singly or in combination have a level of diagnostic strength not greater than 1. The reticulin framework may be lost or overtly reduced as compared to surroundings (level 3 of diagnostic strength for a diagnosis of HCC) but it can also be well preserved. Most pathologists will consider a HGDN in the differential diagnosis. Stromal invasion of well

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Table 2

<table>
<thead>
<tr>
<th>Nomenclature of small hepatocellular lesions</th>
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<tbody>
<tr>
<td><strong>Dysplastic foci (only seen under microscope)</strong></td>
</tr>
<tr>
<td>Microscopic features: cluster of hepatocytes, &lt;1 mm, characterized by small (SCC) or large (LCC) changes.</td>
</tr>
<tr>
<td><strong>Dysplastic nodule</strong></td>
</tr>
<tr>
<td>Gross features: a distinctly nodular lesion that differs from the surrounding liver parenchyma with regard to size, color, texture and degree of bulging of the cut surface.</td>
</tr>
<tr>
<td>Microscopic features: distinguished into two categories:</td>
</tr>
<tr>
<td>1. low grade (LGDN): a (clonal) cell population lacking architectural atypia with mild increase in cellularity as compared to surroundings; portal tracts detectable within;</td>
</tr>
<tr>
<td>2. high grade (HGDN): frank cytological and architectural atypia as compared to surroundings but insufficient for a diagnosis of malignancy; portal tracts detectable within.</td>
</tr>
<tr>
<td><strong>Small HCC</strong></td>
</tr>
<tr>
<td>According to gross and microscopic features it is differentiated into:</td>
</tr>
<tr>
<td>1. early HCC: a vaguely nodular lesion with indistinct margins with a well differentiated histology which may require careful distinction from LGDN; a few portal tracts detectable within;</td>
</tr>
<tr>
<td>2. progressed HCC: a distinctly nodular lesion with well (G1) to moderately (G2) differentiated histology in which malignancy is recognized at first glance; no portal tracts detectable within.</td>
</tr>
</tbody>
</table>
Fig. 1. Features of low grade dysplastic nodule (LGDN). A. LGDN manifesting, at low magnification, as a large and distinct hepatocellular nodule with expansile growth pattern and intranodular portal tracts. B. LGDN (left of dotted line) showing a slight increase in cellularity, compared with the adjacent cirrhotic tissue without architectural atypia. C. LGDN showing occasional foci of large cell changes. D. If correctly biopsied LGDN (as the whole group of dysplastic nodules) stands out over surrounding nodules (dotted lines paralleling all biopsied nodules).

differentiated hepatocytes into portal tract or fibrous septa can be detected and are a very useful diagnostic clue for malignancy (level 3 of diagnostic strength); this histological variable can be substantiated by the use of CK7/19 depicting the ductular reaction which takes place around non malignant nodules but is absent around HCC (loss of DR/CK7/19); as such it has to be looked for in any difficult/doubtful case. Microscopic vascular invasion is not seen.

Small and progressed HCC (HCC of distinctly nodular type or HCC with distinct margins). Progressed HCC may develop from pre-existing dysplastic foci or nodule or from an early HCC. In the latter 2 cases it can take the gross and radiological appearance of the so-called nodule in nodule. At the earliest stage progressed HCC can be small, less than 2 cm, like the well differentiated early HCC. At variance with the latter however, progressed HCC is rarely a diagnostic dilemma either radiologically and histologically, even in the liver biopsy. It is morphologically characterized by a destructive and pushing growth pattern with complete neoarterialization and not uncommon microscopic vascular invasion (25% of the cases). Portal tracts are no longer visualized and the expansile round borders of the tumor are generally rimmed by a condensed fibrosis showing up as a tumor capsule. Histologically small but progressed HCC is well to moderately differentiated (G1/G2), rarely steatotic for more complete and advanced neoarterialization. The average time to recurrence and 5 yr survival are 1.7 years and 48% as compared to 3.9 years and 89% of the early form.

A schematic diagram summarizing the main lesions thought to be involved in the human hepatocarcinogenesis is reported in Fig. 4.

4.1.3. Immunohistochemistry (Fig. 5)

A number of immunomarkers have been recognized as able to selectively label small and well differentiated HCC as compared to non-malignant counterparts. These markers have been originally identified in expression studies targeted to investigate the molecular profile of lesions occurring during hepatocarcinogenesis [10]. Glypican 3 (GPC3), a cell surface heparin sulphate proteoglycan, is an HCC serum and tissue marker with a sensitivity of 77% and specificity of 96%. GPC3 staining must be interpreted in context because it may also be seen in regenerating hepatocytes in a chronic hepatitis setting [8]. Heat Shock Protein 70 (HSP70) is a stress protein implicated in cell-cycle progression, apoptosis and tumorigenesis. It was reported as the most abundantly upregulated gene in early HCC where it can be detected
Fig. 2. Features of high grade dysplastic nodule (HGDN). A. HGDN showing, at low magnification, alternating light and dark eosinophilic areas; intranodular portal tracts (arrows). B. HGDN showing increased cell density, thick trabeculae, rare pseudoglands and small cell changes mainly on the left part of the field (star); no steatosis is detectable; this particular field ask for a distinction from well differentiated HCC. C. Arrows indicate a HGDN in the intranodular liver biopsy; compare with cirrhotic nodules either adjacent or other fragment (dotted arrows). D. In the liver biopsy notice the focal cellular crowding and small cell changes near a portal tract (star).

Using immunocytochemistry in up to 78% of the cases with 95% specificity [8]. Glutamine synthetase (GS) catalyzes the synthesis of glutamine from glutamate and ammonia and it is the main source of energy for hepatocytes located in pericentral areas. However, it is also a target gene of β-catenin, a major driver of human HCC, where GS overexpression is able to mirror β-catenin mutation. In malignant hepatocytes GS immunostaining should be diffuse and strong, a pattern that can be seen in 50% of HCC [8].

In morphologically dubious cases the use of a combination of these markers has been suggested as very useful to increase the diagnostic accuracy, not only in resected specimens but also in the liver biopsy [8,11]. This combination is reminiscent of the overall guide to immunohistochemistry in this challenging field: interpretation of a combination of markers surpasses an individual test. Depending on the technical skills and specific setting applied, any of the markers applied can provide diagnostically useful information. Given that in the foreseeable future the pathologist will be confronted with biopsies from 1–3 cm atypical lesions, one of the most common differential diagnostic questions will be HGDN versus well-differentiated small HCC [12]. In the appropriate clinico-morphological context 2 unequivocal immunomarkers staining (out of 3 among GPC3, HSP70, GS and regardless which one) can detect early and well differentiated HCC in 50% of the cases with 100% specificity (level 3 of diagnostic strength for a diagnosis of malignancy). However, if only 1 out of 3 immunomarkers is positive, a diagnosis of HCC can not be rendered unless the staining is very strong and diffuse but, in these cases, the level of diagnostic strength is 1. Finally, rare HCC are completely negative for these markers.

4.1.4. The histology report

The clinical impact of a correct histopathological diagnosis is detailed in Table 3. Most of the lesions we mentioned can be easily recognized even on the liver biopsy, particularly those lying on the end-terminal of the spectrum (LRN/LGDN and progressed G1–G2 HCC). Comparison with adjacent or extranodular parenchyma and systematic evaluation of morphological features by H&E and reticulin staining is generally able to clearly identify LRN/LGDN and progressed HCC and separate them from the group of HGDN/early HCC. Very challenging may conversely be the distinction between the 2 entities of the latter group, particularly in the liver biopsy. An integrated clinico-pathological approach including the clinical history (cirrhosis in surveillance/previous HCC, oncofetal
Fig. 3. Features of early HCC. A. Gross features of small and early HCC manifesting as a 1.3 cm unencapsulated, vaguely nodular lesion with indistinct margins (arrows). B. A microscopic view of early HCC (bottom right) and the adjacent liver (upper left): a largely unencapsulated steatotic nodule is the most distinguishing feature of the tumor at this magnification. C. At higher magnification the irregular but marked steatosis and the increased cell density raise the suspicion of early HCC; this is confirmed, in this field, by the detection of infiltrating hepatocytes into portal tracts (stromal invasion, arrows). D. Early HCC in this liver biopsy shows up with increased cell density, steatosis and neoarteries (arrows). However this field is not per se diagnostic and requires further studies (reticulin staining and immunomarkers).

markers, number and size of nodules, imaging features, etc.) is recommended in order to put morphology in context. The diagnostic strength and the role of the histological variables for this differential diagnosis are shown in Table 4. Morphology (H&E) alone does not go beyond level 1 of diagnostic strength. The recognition of an extensive neovascularization [detected using the endothelial CD34 marker and the Actin Smooth Muscle Antigen (SMA) for muscolarized unpaired arteries] which is rarely complete in early HCC has a diagnostic strength of 2. Conversely, the following features are able, singly or in combination, to ensure the maximum diagnostic strength (level 3): (a) stromal invasion into portal tracts/septa which can be highlighted by the lack of immunoreactivity for CK7/19; however, stromal invasion can remain difficult to document in the liver biopsy due to the random and unpredictable inclusion of portal tracts into the sample; (b) the partial or total loss of reticulin framework which is, however, not always seen in the earliest phases of malignant transformation; (c) the overexpression as compared to surroundings, of at least 2 of 3 immunomarkers in the neoplastic population (GPC3, HSP70 and GS).

Only a proven diagnosis of malignancy can be definitely rendered on the liver biopsy. Formulations of “suggestive for HCC” are discouraged because biopsy is the last available arrow of clinicians to document a HCC in a cirrhotic setting.
Fig. 4. Illustrations of the main microscopic and macroscopic lesions involved in human hepatocarcinogenesis and putative interrelations.

If that formulation is not possible because of inadequate sampling or too few material available for diagnosis it is better to state that the sampling is not sufficient to make a conclusive diagnostic report (see also Table 5).

By contrast it is obvious that the degree of dysplasia can be affected by sampling. All clinicians involved in this field should be aware of the fact that “a positive biopsy is helpful but a negative biopsy can never be taken as conclusive” [12]. Finally the replacement of biopsy evaluation as the gold standard for the diagnosis of HCC > 2/3 cm does not imply that all atypical cases in a cirrhotic liver represent HCC or its precursors. The difficulties with this assumption [12] include

Table 4

Expected distribution and level of diagnostic strength of histological variables in the differential diagnosis between HGDN and small/early G1 HCC

<table>
<thead>
<tr>
<th>Histological variables</th>
<th>HGDN</th>
<th>Small/early G1 HCC</th>
<th>Diagnostic strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal tract</td>
<td>+</td>
<td>±</td>
<td>Level 1</td>
</tr>
<tr>
<td>Cell density</td>
<td>+</td>
<td>± (up to 1.5–2)</td>
<td>Level 1</td>
</tr>
<tr>
<td>Pseudoglands</td>
<td>±</td>
<td>±</td>
<td>Level 1</td>
</tr>
<tr>
<td>Nuclear Atypia</td>
<td>±</td>
<td>+</td>
<td>Level 2</td>
</tr>
<tr>
<td>Steatosis</td>
<td>–</td>
<td>±</td>
<td>Level 2</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>±</td>
<td>+</td>
<td>Level 2</td>
</tr>
<tr>
<td>Unpaired arteries</td>
<td>±</td>
<td>+</td>
<td>Level 2</td>
</tr>
<tr>
<td>Capillarized sinusoids</td>
<td>±</td>
<td>+</td>
<td>Level 2</td>
</tr>
</tbody>
</table>

| Markers                |      |                   |                     |
| Reticulin loss         | –    | ±                 | Level 3             |
| Stromal invasion       | –    | ±                 | Level 3             |
| At least 2 immunomarkers staining out of 3 | –    | ±                 |

±: may be present; +: usually present; –: absent.

Table 5

Checklist to characterize hepatocellular nodules in biopsy specimens

1. Cut 7 sections, stain one with H&E.
2. Check adequacy on H&E (compare the putative target nodule with surroundings; for this aim an intralesional and extralesional sampling is always recommended).

Not adequate:
Definition: all the cirrhotic nodules are of the same size, look alike and do not contain portal tract (the target nodule can not be distinguished from surroundings or extralesional parenchyma).
Action: repeat biopsy. Second biopsies have shown to increase the sensitivity for HCC detection [3].

Limited:
Definition: the target nodule is clearly discernible but too small/not conclusive for insufficient, necrotic or artifactual cellularity. Action: repeat biopsy if a diagnostically conclusive report can not be obtained.

Adequate:
Definition: the target nodule is clearly discernible; there are three possibilities:
(a) Clearly non-malignant.
Action: try to grade dysplasia (suggestive for low or high grade dysplasia).
(b) Clearly malignant.
Action: mention the histological grade of HCC only in cases of poorly differentiated tumors.
(c) Doubtful.
Action: (i) do reticulin staining and vascular markers (CD34 and/or SMA);
(ii) look for stromal invasion using H&E and CK7/19;
(iii) look for GPC3, HSP70, GS immunoreactivity and overexpression as compared to surroundings.

other lesions that may present in a cirrhotic liver and large regenerative nodules can be >2 cm as described in cirrhosis in autoimmune hepatitis. In addition, another important differential diagnosis is cholangiocarcinoma, which can also be
detected in cirrhosis and whose distinction from HCC will be detailed in the last section of this paper. Mixed HCC/CCA and/or carcinoma of the liver with biphenotypic or progenitor cell expression are not as yet characterized by radiology.

4.2. The sampling and the pathology report of HCC after resection/transplantation

4.2.1. HCC pattern and grading

During progression and size enlargement HCC can develop different clonal populations and an array of well recognized morphological patterns such as trabecular, pseudoglandular, solid/compact, sarcomatoid, scirrhous, etc. Scirrhous HCC should not be confused with fibrolamellar HCC, which is a specific and rare variant occurring in younger patients (20–40 years) and characterized by a desmoplastic lamellar stroma trapping a proliferation of oncocytic-like malignant hepatocytes. HCC patterns can be pure but most often are mixed because of the great heterogeneity of the tumor. Although familiar to pathologists the various patterns to some extent mirror the degree of differentiation of neoplastic cells rather than reflecting separate categories of tumors. For instance, pure pseudoglandular or trabecular forms are mostly well to moderately differentiated (G1–2), while mixed forms are moderately to poorly differentiated (G2–3) and sarcomatoid are undifferentiated (G4). We recommend grading HCC using the Edmondson-Steiner classification or the AFIP modified category (G1–4) because grading is a major issue with independent prognostic significance [13]. Both the Edmondson-Steiner classification and the AFIP classification are based on progressive nuclear features (grade 1: regular nuclei; grade II: some hyperchromatism, nucleoli and $>N/C$ ratio; grade 3: as grade 2 but with more pronounced nuclear irregularity and very prominent nucleoli; grade IV: marked anaplasia with giant and pleomorphic cells). Tumor grading should be formulated in every resected/explanted tumor while its significance in the liver biopsy is more debated, unless poor differentiation is seen. Larger advanced HCC can also be recognized using liver cytology, if clinically required.

4.2.2. HCC growth, spread and vascular invasion

In the early stages most tumors appear to grow as an expanding or encapsulated mass. As tumors enlarge they infiltrate their surroundings and form satellite nodules ($\leq 2$ cm), expression of a peritumoral (within 2 cm) metastatic diffusion close to the main tumor mass. Microscopic vascular invasion is the presence of tumor emboli within vascular spaces rimmed by endothelium and its occurrence is corre-
lated to tumor size. Approximately 50% of larger HCC have microscopic vascular invasion while intrahepatic metastasis via hematogenous spread is found in 60% <5 cm HCC and in 95% >5 cm HCC. Thrombosis of the portal vein occurs in 65–75% of advanced HCC. It has been recently suggested that both the type of embolized vessel (i.e. vessels with a muscular coat documented by SMA immunostaining) and the distance of the same vessels from the main tumor mass, have an independent prognostic significance. More specifically HCC showing either invasion of vessels with a muscular wall and of vessels distant more than 1 cm from the main tumor mass have a very poor outcome [14]. Whether these 2 prognostic parameters should be looked for and reported in every individual HCC has to be determined in prospective studies.

4.2.3. HCC staging; multinodular vs. multicentric HCC (Fig. 6)

Although TNM staging is widely used and available for HCC, most clinical series and treatment decisions are taken using staging systems that incorporate features related to the nature of the underlying liver disease, the functional state of the liver and the size and number of the tumors. The Barcelona Clinic Liver Cancer Staging Classification (BCLC) has gained the widest international consensus and incorporates tumor extent, liver function and overall patient performance status, to stratify individual HCC/patients according to the different available treatments.

Of major importance for obvious clinical implications is the ability to distinguish multinodular tumors into multicentric and metastatic. Multicentric tumors are expected to show a more indolent behaviour with eventual late recurrence (usually not less than 2 years after treatment) while metastatic HCC are more prone to develop early recurrences (usually within 2 years after treatment). To address this issue, which is still very challenging for both clinicians and pathologists, several gross and morphological criteria have been developed (a summary in Fig. 6), although the clinical history and the histopathological features, when available, may not be absolutely accurate. Tumor allelotyping of the nodules has been proposed as the most accurate way to address the issue, but this has still not entered clinical practice [15]. Recommendations for sampling and reporting HCC on tissue explants/resection specimens are summarized in Tables 6 and 7.

5. Hepatocellular carcinoma versus metastatic neoplasms

Metastases are by far the most common malignant neoplasms in non-hepatitic/cirrhotic liver. Without antecedent liver disease, HCC seems to account for about 2% of malignant neoplasms in the liver [16]. However, HCC occurring in liver without or with minimal portal fibrosis may represent up to 20% of the whole burden of HCC [17] and furthermore, although more rarely, malignant tumors arising in another organ can also occasionally metastasize in a cirrhotic liver. Spread to the liver is usually hematogenous. Because of these circumstances and because of the wide spectrum of histologic differentiation with a great diversity of appearance of HCC, the differential diagnosis between HCC and other tumors involving the liver can be difficult and even more challenging in core and fine-needle biopsies.

Lung, colon, pancreas and breast are the most common primary sites of hepatic metastases [18], but malignant tumors from almost any site can metastasize to the liver. Moreover, while some tumors such as melanoma, neuroendocrine carci-
Table 6
Handling of HCC in surgical explants/resections

Native liver explants:
Weigh and serially section the cirrhotic liver every 5–7 mm; check and longitudinally open the hepatic artery, portal vein, bile duct and gallbladder. Sample margins from hepatic vessels and bile ducts, lymph nodes and any obvious intraluminal lesions such as strictures, vegetations, tumors and thrombi. Take tissue blocks from the right lobe (at least 5), left lobe (at least 3), caudate (at least 1) and hilum (at least 3: one parallel to the resection margin, and the others perpendicular to the right and left side of the deep hilum); some sections must include Glisson’s capsule. Take at least one block every 1 cm of the main tumor including satellites. Measure and sample every nodule standing out in the surroundings for colour, texture and consistency. Make a drawing to record any obvious lesions.

For diagnostic proposes all specimens are formalin fixed, paraffin embedded and H&E stained. Special stains as appropriate in selected blocks. Special procedures for handling tissue (frozen sections, immediate bulk freezing of tissue) are recommended for additional studies and tissue banking.

Resection:
Describe type of resection and involved segments. Measure and sample every single tumor including capsule, satellites and tumor. Measure the distance of the tumor from the proximal margin (wide margins if >1 cm).

Table 7
Checklist for HCC reporting (resection/explants)

Tumor:
Size, number, macroscopic and microscopic vascular invasion, capsule, grading, satellite nodules, multicentric vs. metastatic disease (if feasible) and pathological staging (AJCC, American Joint Committee of Cancer staging criteria/TNM system). Resection margins. Evidence of previous chemoembolization or radiofrequency ablation. Associated not-malignant nodules.

Background:
Underlying hepatic disease with staging and grading as appropriate.

Fig. 7. A. Hep Par 1 positivity in HCC cells with characteristic granular staining. B. Distribution of CK20/CK7 phenotype in adenocarcinomas of different organs. C. Cytokeratin 7 and 20 expression in cholangiocarcinomas varies along the biliary tract; the sensitivity of CK7+/CK20− profile for CC is higher in peripheral tumors than in nonperipheral CC (for detailed text see Rullier et al. [20]).

5.1. Commonly used markers for HCC diagnosis

5.1.1. Hepatocyte paraffin 1 (Hep Par 1)
Hep Par 1 is a monoclonal antibody against an urea cycle enzyme, carbamoyl phosphate synthetase 1 (CPS1), located in mitochondria. It has emerged as the most sensitive (around 80% to 100%) and specific immunohistochemical marker for HCC and reflects hepatocyte differentiation in hepatic as well as extra-hepatic tumors. Hep Par 1 yields a diffuse cytoplasmic granular staining pattern in normal and neoplastic hepatocytes, sparing bile duct epithelium and inflammatory cells (Fig. 7A). However, not all HCC stain uniformly and variation in staining may occur, particularly when there is fatty, clear cell or oncocytic change and hence needle biopsies can be negative. Hep Par 1 positivity is also probably related to the differentiation grade of the hepatocellular
tumor, ranging from nearly 100% in well-differentiated (WD) HCC to <50% in poorly differentiated (PD) or sclerosing HCC. Although rather specific for hepatic differentiation, Hep Par 1 can be focally expressed by non hepatic tumors, as adenocarcinoma of lung, oesophagus, stomach, gallbladder, small intestine, adrenal gland, melanoma, paraganglioma [19]. Adenocarcinoma with hepatoid differentiation may also focally express Hep Par 1.

5.1.2. Polyclonal carcinoembryonic antigen (pCEA)

CEA is a glycoprotein present in the glycocalix of fetal epithelial cells and in small amounts in normal adults cells. This antibody stains a CEA-like cross-reactive substance called biliary glycoprotein present in bile canaliculi and ductal epithelium but not in hepatocytes. Diffuse cytoplasmic expression is observed in most adenocarcinomas (>90%). In HCC, pCEA staining reveals a characteristic and specific “chicken-wire fence” canalicular pattern, not observed in adenocarcinomas. However, as already noted for Hep Par 1, the sensitivity of this marker given by the canalicular staining, decreases with increasing anaplasia, ranging from from 60% to 95% for WD and moderate differentiated HCC to 25–50% of PD HCC. Monoclonal CEA is not reactive in HCC.

5.1.3. Alpha-fetoprotein (AFP)

AFP is an oncofetal protein expressed mainly in fetal gut, liver and yolk sac. Its expression in a tumor is specific for hepatocellular differentiation if germ cell tumors can be excluded. Normal livers do not express AFP. Staining tends to be focal and sensitivity is about 30%. Interestingly, AFP expression seems to be more frequently in PD-HCC (45%) compared with low-grade tumors (18%). In the experience of the authors, this antibody may be technically problematic and difficult to interpret because of background.

5.1.4. Common Acute Lymphoblastic Leukemia Antigen (CD10)

CD10 is a zinc-dependent 90 to 110 kDa cell membrane metalloproteinase normally expressed in several normal tissues, including kidney, liver, small intestine, placenta, gonads, adrenal gland and brain. CD10 in HCC typically shows a canalicular pattern similar to that of pCEA, not seen in adenocarcinoma, but displaying a lower sensitivity (50%) compared to pCEA.

5.1.5. Negative stain: MOC-31

MOC-31 is a monoclonal antibody that reacts with

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Most useful panel of antibodies</th>
<th>Typical profile and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC vs. renal cell carcinoma (RCC)</td>
<td>Hep Par 1, pCEA, RCC Ag, PAX-2</td>
<td>HCC: Hep Par 1+, pCEA+, RCC Ag+, PAX-2; RCC: Hep Par 1–/PAX-2+, RCC Ag+, PAX-2; Additional RCC marker: vimentin; Additional HCC markers: GPC3, CD10</td>
</tr>
<tr>
<td>HCC vs. neuroendocrine carcinoma (NE)</td>
<td>Hep Par 1, pCEA, Synaptophysin (Syn), Chromogranin (CG), MOC-31</td>
<td>HCC: Hep Par 1+, pCEA+, Syn+, CG+, MOC-31–; NE: Hep Par 1–, pCEA–/Syn–, CG–, MOC-31+; CDX2 and TTF-1 can be used for identification of primary site of NE</td>
</tr>
<tr>
<td>HCC vs. adrenocortical carcinoma (ACC)</td>
<td>Hep Par 1, pCEA, Inhibin, Melan-A</td>
<td>HCC: Hep Par 1+, pCEA+, Inhibin–, Melan-A–; ACC: Hep Par 1–, pCEA–, Inhibin+, Melan-A+</td>
</tr>
<tr>
<td>HCC vs. melanoma</td>
<td>Hep Par 1, pCEA, HMB-45, Melan-A, S-100</td>
<td>HCC: Hep Par 1+, pCEA+, HMB-45+, Melan-A+, S-100–; Melanoma: Hep Par 1–, pCEA–, HMB-45–, Melan-A–, S-100+; Not useful marker: GPC3</td>
</tr>
</tbody>
</table>

Table 8
Role of immunohistochemistry in the differential diagnosis HCC vs metastasis [21]

Levels of certainty/probability: “Highly suggestive”
HCC: Hep Par 1+, pCEA+, MOC-31–; MA and CC: Hep Par 1–, pCEA–/MOC-31+; Additional HCC markers: GPC3, CD10; Additional MA and CC markers: CK7/CK20, site specific markers like TTF-1 (lung, thyroid), CDX2 (colon, small intestine, ampulla Vater), PSA (prostate), ER and Pr (breast and endometrium); Not useful markers: CK19, AE1/AE3 and CAM 5.2.

Diagnostic strength: level 3

Levels of certainty/probability: “Suggestive”
HCC: Hep Par 1+, PAX-2–, RCC Ag–; RCC: Hep Par 1–/PAX-2+, RCC Ag+; Additional RCC marker: vimentin; Additional HCC markers: GPC3 and pCEA.

Diagnostic strength: level 2

Levels of certainty/probability: “Highly suggestive”
HCC: Hep Par 1+, pCEA+/Syn–, CG–, MOC-31–; NE: Hep Par 1–, pCEA–/Syn–, CG–, MOC-31+; CDX2 and TTF-1 can be used for identification of primary site of NE.

Diagnostic strength: level 3

Levels of certainty/probability: “Suggestive”
HCC: Hep Par 1+, pCEA+, Inhibin–, Melan-A–; ACC: Hep Par 1–, pCEA–, Inhibin+, Melan-A+.

Diagnostic strength: level 2

Levels of certainty/probability: “Highly suggestive”
HCC: Hep Par 1+, pCEA+, HMB-45+, Melan-A+, S-100–; Melanoma: Hep Par 1–, pCEA–, HMB-45–, Melan-A–, S-100+; Not useful marker: GPC3.

Diagnostic strength: level 3

Levels of certainty/probability: “Diagnostic”
Cave: Angiomyolipomas do also express melanoma markers. However SMA, CK and CD117 can also be expressed.
membrane glycoproteins expressed in benign and malignant glandular epithelia of multiple sites, but not in lymphomas, melanomas and HCC. 50% to 98% of cases of adenocarcinoma stain with a cytoplasmic pattern.

5.1.6. Cytokeratins

Normal and neoplastic hepatocytes express cytokeratins (CK) 8 and 18 and about 70% of HCC are negative for CK7, CK19, and CK20. Therefore, CK7 and CK20 are more helpful in determining the primary site once the diagnosis of adenocarcinoma has been established (Fig. 7B and C) [20].

An immunohistochemical approach in the differential diagnosis of HCC is shown in Table 8.

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Conflict of interest

The authors have no other conflict of interest to report.

References