Hepatocellular carcinoma: a clinical and pathological overview

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Summary
HCC incidence rates have been rising in the past 3 decades and by 2025 > 1 million individuals will be affected annually. High-throughput sequencing technologies led to the identification of several molecular HCC subclasses that can be broadly grouped into 2 major subgroups, each characterized by specific morphological and phenotypical features. It is likely that this increasing knowledge and a more appropriate characterization of HCC at the pathological level will impact HCC patient management.

Key words: hepatocellular carcinoma, tumor microenvironment, angiogenesis, prognosis, diagnosis

Clinical background

Epidemiology. HCC incidence rates have been rising in the past 3 decades and similar trends are expected through 2030. The WHO stated in 2015 that HCC was the fifth most common cancer worldwide and the second most common cause of cancer-related death. The global observatory on cancer reported in 2018 liver cancer was the sixth most common cancer world-wide, with 841,080 new cases, and the fourth leading cause of cancer-related death globally. By 2025, > 1 million individuals will be affected by liver cancer annually. Over 90% of HCC cases occur in the setting of chronic liver disease. Cirrhosis from any etiology is the strongest risk factor for HCC. Several important risk factors are related to HCC, among these HBV and HCV chronic infection and NAFLD/NASH play the major role. About 3.5% of the global population, 257 million persons, are chronically infected with HBV. With the use of direct-acting antiviral (DAA) therapy, patients with HCV infection have been successfully treated to achieve a sustained virological response and this has resulted in a 50-80% reduction in the risk of HCC. The lifetime risk of these persons to develop cirrhosis and/or HCC is 15% to 40%. In endemic areas, HBV is etiologically implicated in as many as 50% to 80% of all HCC cases, a figure decreasing to 20% in western countries. HCV infection affects 71 million persons all over the world. With the use of direct-acting antiviral (DAA) therapy, patients with HCV infection have been successfully treated to achieve a sustained virological response and this has resulted in a 50-80% reduction in the risk of HCC. Nonetheless, HCV chronic infection is a major contributing factor to liver cancers in the USA, and is associated with 50% of cases. NAFLD/NASH has a global prevalence of 24%, with the highest rates reported in South America and the Middle East (30-35%). It has been estimated that up to 25% of NAFLD can progress to NASH and that up to 20% of pa-
patients with NASH have cirrhosis. NASH-associated cirrhosis carried a 2.4% to 12.8% increased risk of HCC. Several studies have demonstrated that 25-30% of NASH-associated HCC occur in the absence of cirrhosis. Since 2010, the proportion of HCC attributed to NASH has rapidly increased, currently representing 15-20% of cases in the West.

**Diagnosis.** Imaging plays a critical role in HCC diagnosis. HCC lesions are brighter than the surrounding liver in the arterial phase in a CT scan or MRI and less bright than the surrounding parenchyma in the venous and delayed phases, and this is due to the differential blood supply of the tumor compared with the background liver. This phenomenon of ‘arterial enhancement and delayed washout’ has a sensitivity of 89% and a specificity of 96% for HCC and is regarded as the radiological hallmark of HCC. In patients with liver cirrhosis the presence of these typical vascular hallmarks identified by quadruple-phase CT or dynamic contrast-enhanced MRI is sufficient for diagnosis without requiring histological confirmation. A more recent radiological approach, the LI-RADS (Liver Imaging Reporting and Data System) assigns lesions > 10 mm to different categories reflecting the relative probability of the lesion being benign, HCC, or other hepatic malignant neoplasm according to an enlarged the number of criteria (arterial phase enhancement, tumor size, washout, enhancing capsule and threshold growth features). AFP and other serum biomarkers generally have a minor role in the diagnosis of HCC.

**Treatment.** Therapeutic options might be limited because of the patient’s overall health status (cirrhosis). Nonetheless, there have been significant advances in HCC treatment over the past 10 years. Some approaches offer the chance of long-term response: they include surgical resection, orthotopic liver transplantation (LT), and ablative techniques such as thermal ablation. Other therapies attempt slowing tumor progression and include transarterial chemoembolization (TACE), transarterial radioembolization (TARE), stereotactic body radiation therapy (SBRT), and systemic chemotherapy. The most appropriate treatment should be indicated by a multidisciplinary approach taking into consideration different patients features and stratifying them in a disease stage. Currently, HCC is treated according to the Barcelona Clinic Liver Cancer scheme, which stratifies patients into five categories: very-early (stage 0), early (stage A), intermediate (stage B), advanced (stage C) and terminal stage (stage D). The BCLC staging system links tumor stage, liver function, cancer-related symptoms and performance status to an evidence-based treatment algorithm.

In general, surgical resection or liver transplantation is the first option to treat early-stage HCC yielding the best outcomes, with a 5-year survival of ~70-80%. Resection should be offered to patients who have a single tumor (regardless of size), well-preserved liver function (Child-Pugh A with total bilirubin < 1 mg/dl), no signs of portal hypertension (varices or ascites) or a hepatic venous pressure gradient (< 10 mmHg), and a preserved performance status. LT should follow Milan criteria: a single tumor of 5 cm or up to three nodules of 3 cm with no evidence of macrovascular invasion or extra-hepatic manifestations. The recurrence of HCC after hepatic resection rates as high as 70% at 5 years, even in patients with a single tumor ≤ 2 cm. Recurrences can be divided into either early (2 years), and late (> 2 years): the former likely representing the result of metastatic spread; the latter de novo tumors arising in a microenvironment predisposed to carcinogenesis. The 10-year recurrence rate after transplantation is 10-15% for HCC tumors within Milan criteria and 20% in those down-staged to the Milan criteria. In very early-stage disease (tumors < 2 cm diameter), thermal ablation has demonstrated similar outcomes to surgical resection and thus may be recommended as first-line treatment, specifically in light of its lesser invasiveness and morbidity compared with surgery. Radiofrequency ablation and percutaneous ethanol injection are effective for small tumors, but radiofrequency ablation is superior for tumors larger than 2 cm vs percutaneous ethanol injection. Adjuvant therapies to be used after the curative approaches, are an unmet medical need, as randomized controlled trials have so far yielded negative results. For intermediate-stage HCC, transarterial chemoembolization (TACE) has been the most widely used treatment and the standard of care over the past two decades. TACE is recommended for patients who do not have vascular invasion or extrahepatic spread and are not candidates for liver transplantation, surgical resection, or local ablation because of large tumor size or multifocal tumor, with an estimated average of median overall survival of ~30 months. Transarterial radioembolization (TARE) has shown efficacy in phase II investigations but has not been established as a primary standard of care by guidelines. Approximately 50-60% of patients with HCC, mostly at advanced stage, will be treated with systemic therapies. This field has seen significant progress in the past 5 years. Until 2017 sorafenib was the only available standard of care for advanced HCC. In 2018 a phase II study demonstrated the efficacy of lenvatinib, which was then approved for advanced-stage HCC in the first-line setting. In case of progression
to single-agent regimens, regorafenib, cabozantinib, and ramucirumab showed improved survival benefits and were therefore approved as second-line treatment. The median survival for these treatments was 8-10 months: a figure more than doubled by the recent approved combination of atezolizumab (anti-PDL1 antibody) and bevacizumab (anti-VEGF antibody).

**Pathology**

**Gross features.** Single HCC can be classified as “Vaguely nodular” (a nodule with indistinct margins), “Expanding nodular” (a round expansive nodule with a distinct margin), “Multinodular confluent” (cluster of small and confluent nodules), “Nodular with perinodular extension” (extranodular growth in < 50% of the circumference) and “Infiltrative” (extranodular growth in > 50% of the tumor circumference). Single HCC with nodular morphology have favorable outcomes compared with those with multinodular, perinodular or infiltrative growth patterns. In up to 30% of cases, HCC might present with multiple, clearly separated, tumors. In this case, the number of lesions should be recorded and each lesion should be described in detail. A satellite nodule is a small nodule close (< 2 cm) to the main tumor.

**Microscopic and molecular features.** HCC develops from a cirrhotic liver through a multistep sequence. This latter includes pre-neoplastic lesions, represented by low-grade (LG-) and high-grade (HG-) dysplastic nodules (DN) and early neoplastic lesions, represented by early HCC (eHCC) and small and progressed HCC (pHCC). Pre-neoplastic and early neoplastic lesions are characterized by progressive accumulation morphological and molecular abnormalities. The former are represented by a wide spectrum of findings including increasing cell density and nuclear-to-cytoplasmic ratio; loss of reticulin framework; presence of unpaired arteries and pseudo-gland formation. At the molecular level, HG-DN, eHCC and pHCC are characterized by a progressive increase of TERT promoter mutation as a unique fingerprint. As shown in Table I, however, none of these alterations is sufficient, per se, to distinguish lesions staying close at the border between dysplasia and malignancy. To this aim, the international guideline recommends the use of a panel of immunohistochemical markers. In more advanced lesions, architectural alterations and cytological changes are overt and diagnosis of malignancy is not under discussion. In this setting the differential diagnosis might involve cancer with mixed hepatocellular and cholangiocellular differentiation or metastasis (unusual in the setting of a cirrhotic liver). HCC histological subtyping rests on the evaluation of architectural growth patterns (microtrabecular, macrotabecular, pseudo-glandular, compact) and cytological aspects (clear cell, fatty change, cholestasis, pleomorphic cells, spindle cells). It should be observed, however, that several different features frequently coexist in the same lesions. HCC differentiation is graded into four grades according to the Edmondson and Steiner classification and into three according to the WHO.

Studies based on high-throughput sequencing led to the identification of several molecular HCC subclasses. Regardless of the nomenclature used, HCC can be divided into 2 major subgroups (Fig. 1). The non-proliferation class, characterized by chromosomal stability and frequent TERT promoter mutations. These HCCs are less aggressive and show well to moderate histological differentiation, less frequent vascular invasion and low levels of AFP. They are related to non-alcoholic and alcoholic steatohepatitis and HCV infection. Two distinct subgroups characterized this class: the WNT-β-catenin/CTNNB1-mutated subclass which drives an immune-excluded phenotype with low immune infiltration, and the interferon subclass which presents a highly activated IL6-

| Features            | HGDN     | eHCC     | Discriminatory value |
|---------------------|----------|----------|----------------------|
| Portal tract        | +        | ±        | Low                  |
| Cell density        | + (up to 1.5-2) | + (x 2 or more) |                      |
| Pseudoglands        | ±        | ±        |                      |
| Nuclear Atypia      | ±        | +        |                      |
| Steatosis           | -        | ±        |                      |
| Unpaired arteries   | ±        | +        | Medium               |
| Reticulin loss/decrease | ±        | ±        |                      |
| TERT promoter mutation | ±        | +        |                      |
| Stromal invasion    | -        | ±        | High                 |
| 2 markers staining out of 3 | -       | ±        |                      |
JAK-STAT signaling pathway, with a more inflammatory tumor microenvironment. The other major class of HCC, i.e. proliferation-class, is characterized by high chromosomal instability, global DNA hypomethylation, frequent TP53 mutations, and overexpression of genes involved in the cell cycle. These HCCs are more aggressive and show poor histological differentiation, high vascular invasion and increased levels of AFP. The proliferation-class can further be divided into two subclasses. The former, i.e. S1 or iCluster 3, shows Wnt-TGFβ activation and immune-exhausted phenotype and is barely recognizable at morphology; the latter, S2 or iCluster 1, displays a progenitor-like phenotype, highlighted by the expression of stem cell markers (CK19, EPCAM) and is characterized by activated IGF2 and EPCAM signaling pathways.

According to the last WHO classification of liver tumors, about 1/3 of all HCC can be classified into specific subtypes: steatohepatitic, clear cell, macrotrabecular massive, scirrhous, chromophobe, fibrolamellar, neutrophil- and lymphocyte-rich. In the following section we will illustrate some of these entities.

CTNNB1 MUTATED (CHOLESTATIC-) HCC (FIG. 2 A, B)

HCCs with mutations in CTNNB1 are well-differentiated tumors, characterized by microtrabecular and pseudoglandular patterns, intratumor cholestasis and lack of immune infiltration. CTNNB1 encodes β-catenin, a key intracellular transducer of the Wnt signaling pathway that regulates liver physiology and zonation. Mutations result in β-catenin stabilization and subsequent nuclear accumulation, where it enhances cell proliferation and survival. HCCs with mutations in CTNNB1 are characterized at phenotypical level by glutamine synthetase (GS) and nuclear β-catenin expression. Interestingly, diverse mutations have been correlated with different staining patterns. These tumors are characterized by the expression of genes involved in hepatocellular differentiation and function, such as APOB, ALB, HNF1A or HNF4A, and by the dysregulation of bile salt transporters which contribute to their cholestatic phenotype. One of these transporters, SLCO1B3, is responsible for the uptake of the MRI contrast agent gadoxetic acid.
**Macro trabecular-massive HCC (MTM-) HCC**

MTM-HCC are tumors characterized by a macrotrabecular (> 6 cells thick) growth pattern in > 50% of the lesion, regardless of the associated cytological features. They exhibit a very aggressive phenotype, with frequent satellite nodules and vascular (micro and/or macro) invasion. It frequently occurs in patients infected by HBV and with high alpha-fetoprotein serum levels. At the genetic level, MTM-HCC often harbors TP53 mutations and/or FGF19 amplification and they are characterized by angiogenesis activation, with angiopoietin 2 and vascular endothelial growth factor A (VEGFA) overexpression. Angiopoietin 2 is responsible for the destabilization of established blood vessels and subsequent vascular sprouting. It also disrupts interactions between endothelial and peri-endothelial cells, which results in an increased sensitivity to VEGFA.

**Scirrhou HCC (Fig. 2E)**

The scirrhous subtype is characterized by an admixture of abundant dense stroma and neoplastic cells. These latter frequently express, at phenotypical level, markers of progenitor or cancer stem cells, including CK7, CK19, or CD133. Accordingly, it has been put forward the hypothesis that these tumors have an intermediate molecular trait, between HCC and cholangiocarcinoma. Consistent with its histological appearance, scirrhous HCC are characterized by the activation of TGF-β pathway, with overexpression of VIM, SNAIL, SMAD4 and TWIST and features of epithelial-to-mesenchymal transition.
This subtype is characterized by inflammatory infiltrates, cell ballooning, peri-cellular fibrosis and Mallory-Denk bodies. At phenotypical level neoplastic cells show overexpression of C-reactive protein (CRP) - a target gene of JAK/STAT signaling. These tumors are often well-differentiated and they associated with gene expression profile similar to that of non-tumor liver.

LYMPHOCYTE-RICH HCC (FIG. 2G)

The latest WHO defined these HCC as lesions with lymphocytes outnumbering neoplastic cells in most fields at on H/E. Nonetheless a consensus definition on the cut-off value for intratumor lymphocyte density is currently lacking. This rare variant has been associated with improved overall survival, supporting the hypothesis that the lymphocytic infiltrate plays an antitumor effect. The lymphocytes show a predominance of cytotoxic CD8+ elements, with increased programmed cell death 1 ligand 1 (PD-L1) and programmed cell death 1 (PD1) expression. As opposite to other settings, such as colon and lung cancer, where lymphocytic infiltration has been linked with microsatellite instability and/or high mutational, none of these HCCs were microsatellite instable or associated with a higher number of somatic mutations.

Figure 2. Pathology of HCC. (E) Scirrhous. This HCC is characterized by abundant stroma separating neoplastic trabeculae. (F) In this HCC, neoplastic cells are characterized by diffuse steatosis and occasional cell ballooning; the tumor shows scattered inflammatory infiltrates. (G) Lymphocyte-rich. A rich intratumoral infiltrate of lymphocytes denotes this HCC. (H) A clear cell variant of HCC.

COMBINED HEPATOCELLULAR-CHOLANGIOCARCINOMA

A subset of primary liver cancer may exhibit both hepatocytic and biliary differentiation. These biphenotypic tumors are much rarer, accounting for less than
5% of all liver cancers. They were first described in 1903 by Gideon Wells and, since then, the definition of this entity has changed several times. Recently an international group proposed a consensus terminology later accepted by the WHO. It was recommended to call these lesions as combined hepatocellularcholangiocarcinoma (cHCC-CCA) and the diagnostic criteria proposed was the unequivocal presence of both hepatocytic and cholangiocytic differentiation within the same tumor on routine H&E. Immunohistochemical markers of hepatocytic (HAS, Arginase, CD10 and polyclonal CEA) and cholangiocytic (CK7, CK19) differentiation may help, but is neither necessary nor sufficient.

A recent study showed that HCC and CCA components had very similar global gene expression profiles, thus suggesting a monoclonal origin. The most frequently mutated driver genes were TP53 (49% of the cases), TERT promoter (23% of the cases), AXIN1 (10% of the cases), and KMT2D (9% of the cases), mutations that may be associated with either HCC or ICCA. In the same study, it was also suggested that nestin might serve as a biomarker for the diagnosis and prognosis of cHCC-ICC.

**VETC (Fig. 3)**
The availability of tissue biomarkers remains an unmet need for HCC management. Some of the above-mentioned histopathological subtypes, MTM-HCC in particular, had been proposed as predictors of prognosis, but not validated in external cohort. By contrast, a robust prognostic significance has been proposed for a peculiar vascular phenotype, characterized by CD34+ vessels encapsulating tumor cluster (VETC). VETC might be present in up to 40% of HCC and associates with higher attitude of tumoral cells to infiltrate vessels. Interestingly, a recent study demonstrated that this phenotype predicts the response to sorafenib.

**Real-life diagnostic issues**
In the daily practice, the pathologist’s diagnostic involvement is restricted to specific clinical settings.

**LIVER BIOPSY IN PATIENTS WITH HEALTHY LIVER: DIAGNOSTIC OF WELL DIFFERENTIATED HEPATOCELLULAR LESIONS**
In this setting the differential diagnosis should take into consideration a benign liver lesion, mostly represented by focal nodular hyperplasia (FNH) and hepatocellular adenoma (HA) and atypical HA or/and HCC. The first question is whether the lesions has been adequately

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**Figure 3. Vascularization of HCC.** (A, B, C) The images illustrate an HCC (lower right corner, A) with a rich vascular support as highlighted by a CD34 staining (B); at higher magnification CD34+ vessels show a capillary distribution; (C). (D, E, F) also this HCC (right part, D) shows a rich vascular network when stained with CD34 (E); in these cases however, CD34+ vessels encapsulate clusters of neoplastic cells (F) featuring a peculiar phenotype described as VETC.
sampled: both FNH and HA may have a subtle and deceptive morphology as to that their borders may be difficult to be clearly localized. A good tool to highlight them, particularly in HA, is an endothelial cell marker (we use CD34) which will permit in the majority of the cases to discern the profile of the punched lesion. Once the lesion has been identified we usually evaluate whether pseudo-portal tracts (fibrous tissue with arteriolar vascular structures and ductular reaction), can be documented, at H/E or using a CK7, within the lesion. The presence of pseudo-portal tracts suggests two diagnostic alternatives: a) FNH, b) Inflammatory-HA (I-HA). FNH and I-HA may be distinguished in most of the cases using a panel GS, CRP and Serum Amyloid Alfa (SAA). The profile GS\textsuperscript{map-like}/SAA\textsuperscript{-}/CRP\textsuperscript{-} supports FNH while GS\textsuperscript{-}/SAA\textsuperscript{+}/CRP\textsuperscript{+} or GS\textsuperscript{+}/non-map-like/SAA\textsuperscript{+}/CRP\textsuperscript{+} support I-HA. Indeed, GS immunoreactivity characterize those I-HA with an activation of β-catenin pathway. If pseudo-portal tracts are not detectable inside the lesion and GS staining is completely negative, there are two alternatives: a) steatotic-HA and b) usual-HA (u-HA). The evidence of steatosis favor a diagnosis of steatotic-HA (S-HA) and lack of expression of Liver Fat-ty Acid Binding protein (LFABP) in tumoral hepatocytes will prove it. A non-steatotic and non-atypical adenoma with a LFABP\textsuperscript{-}/SAA\textsuperscript{-}/CRP\textsuperscript{-}/GS\textsuperscript{-} phenotype should be classified as u-HA. Finally, when the lesion lacks pseudo-portal tracts, inflammatory/teleangectatic morphol-
ogy (SAA/PCR) and shows atypical hepatocytes and GS expression the nodule is likely to be a β-catenin-HA variant. GS antibody, when the staining is strong and diffuse, shows the highest diagnostic accuracy (absolute specificity and sensitivity).

Liver biopsy, in patients with hepatitis/cirrhotic liver: diagnostic of well differentiated hepatocellular lesions

In this setting the differential diagnosis rests between HGDN and eHCC. Morphological features suggested as useful in this differential diagnosis include the decrease of reticulin framework, the presence of stromal invasion, and the progressive transition toward CD34 expression by endothelial cells (Tab. I). However, none of these can, per se, objectively separate HGDN from eHCC. Rather, the use of a panel of markers, namely glypican 3 (GPC3), heat shock protein 70 (HSP70), and GS warrants 100% specificity, with a sensitivity of 49% \(^{74,75}\). Sensitivity that can be increased to 64% by introducing a further marker Clathrin Heavy Chain (CHC) \(^{76}\). On the other hand, the pathologist should be aware that the use of single marker alone can be misleading and should not be considered as a proof of malignancy. GPC3 immunoreactivity can be observed in a few cirrhotic cells and in up to 10% of cells of HGDN. HSP70 is normally expressed by apoptotic hepatocytes, isolated perisepetal hepatocytes, and stellate cells. Finally, GS shows peculiar pattern of staining according to different clinico-pathological conditions \(^{77}\).

Liver biopsy, in patients with hepatitis/cirrhotic liver: diagnostic of poorly differentiated lesions

In this setting the differential diagnosis rests between a primary liver cancer (HCC, CC and combined HCC-
CC) and a metastasis. As already observed the great majority of bona fide HCC are diagnosed according to radiological criteria and treated accordingly. Some of these patients underwent a liver biopsy after standard treatments (surgical, ablative and medical) and before enrolment in clinical studies. These cases, due to their natural history and treatment as well, are poorly differentiated, partially necrotic, or even shifted toward a stem differentiation. Nonetheless, to be considered eligible for the study they need a conclusive histopathological diagnosis. In this setting the pathologist might benefit from a few immunohistochemical markers to prove the hepatocytic differentiation of the lesion. These include HepPar-1, Arginase-1, CD10, pCEA, GPC3 and BSEP. Table II illustrates the performances of the each of these histotype-markers when used alone 78.

**Liver resection: essential criteria**

Clinical and radiological features predicting HCC outcome are part of the current staging system, including the BCLC scheme, mentioned above. This information should be integrated and completed, by pathological features when a resection is performed. The essential pathological criteria that should be reported are represented by the gross evaluation of tumor size and number and by the microscopic evaluation of tumor type and grade, vascular invasion and the expression

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**Figure 6. Diagnosis of well differentiated hepatocellular lesions in healthy liver: teleangiectatic/inflammatory Hepatocellular Adenoma.** (A) This liver biopsy illustrates a well differentiated hepatocellular lesion characterized, at higher magnification, by teleangiectatic vessels; (B) CK7 highlights that the lesion is characterized by the presence of scattered pseudoportal tracts; (C and D) the lesion is immunoreactive for SAA and PCR. The morpho-phenotypical findings are conclusive for telenangiectatic HA.
of CK19. The correct definition of the histotype enriches the pathological report with prognostic information. The MTM histotype, has a poorer outcome while the lymphocyte-rich HCC has a better prognosis. The histotype might also be used as a predictive evidence. It has been shown that lymphocyte-rich HCC are sustained by the presence of an active immune infiltrate which makes at least questionable the use of drugs designed to restore the immune function such as immune check point inhibitors. On the other hand, HCC correlated to β-catenin pathway activation are associated with a poor immune infiltrate and likely do not represent the target for drugs affecting lymphocytes function. HCC grading systems (Edmondson-Steiner and WHO, with several “home-made” variations) strongly predict patient outcome in liver resection or transplantation, with the worst grade driving HCC prognosis. Accordingly, a clinical meaningful pathological report should indicate the predominant and also the worst grade (in line with what is done for prostatic biopsy). Microscopic vascular invasion (MVI) is a major prognostic feature of HCC and is associated with advanced tumor stage, distant metastasis and adverse outcome. MVI occurs at the rates of 25%,

**Figure 7.** Diagnosis of well differentiated hepatocellular lesions in healthy liver: Atypical Hepatocellular Adenoma. (A) These biopsies document a well differentiated hepatocellular lesion (*) and the surrounding parenchyma; (B) the lesion, which is barely seen on H/E at scanning magnification, is highlighted and clearly outlined by CD34; and shows (C) strong and diffuse immunoreactivity for GS; (D) at higher magnification, the lesion is characterized by a mild degree of architectural disarrangement and cytological atypia (pseudo-glands and increased N/C ratio). (E) reticulin framework is conserved and (F) few neoplastic cells showing nuclear immunoreactivity for HSP70. The morpho-phenotypical findings are conclusive for an atypical HA.

**Table II.** Sensitivity of markers used to demonstrate HCC in a liver lesion.

| Marker | All HCC, sensitivity: Best performance | All HCC, sensitivity: Worst performance | G3 HCC, sensitivity |
|--------|---------------------------------------|----------------------------------------|-------------------|
| HepPar1 | 84%                                  | 70%                                    | 22-78%            |
| Arginase | 96%                                  | 84%                                    | 44-89%            |
| BSEP    | 90%                                  | -                                      | 78%               |
| pCEA    | 81%                                  | 45%                                    | 78%               |
| CD10    | 74%                                  | 50%                                    | 67%               |
| GPC3    | 54%                                  | -                                      | 67%               |
40%, 55% and 63% in HCC < 3, 3-5, 5-6.5, and > 6.5 cm. Accordingly immunohistochemical markers surrogate of MVI are intensively investigated. Finally, the use of stemness-related biomarkers represents the field where the translation of molecular information on the clinical practice is more advanced. Several stemness-related markers have been identified and intensively investigated (CK19, EpCAM, CD133, SALL4, NCAM, OV6, CD90, nestin, CD44) and almost all were associated with a more aggressive clinical behavior. In particular HCCs with CK19 immunostaining in > 5% of tumor cells show higher recurrence rates and higher rates of lymph node metastasis.

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