PATHOLOGICA 2021;113:203-217;
DOI: 10.32074/1591-951X-295

Review

Hepatocellular carcinoma: a clinical and pathological overview

Salvatore Lorenzo Renne[1,2], Samantha Sarcognato[3,4], Diana Sacchi[3], Maria Guido[3,4], Massimo Roncalli[1,2], Luigi Terracciano[1,2], Luca Di Tommaso[1,2]

[1] Department of Biomedical Sciences, Humanitas University, Milan, Italy; [2] IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy; [3] Department of Pathology, Azienda ULSS2 Marca Trevigiana, Treviso, Italy; [4] Department of Medicine - DIMED, University of Padova, Padova, Italy

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

Received and accepted: April 26, 2021

Correspondence
Luca Di Tommaso
Department of Pathology, IRCCS Humanitas Research Hospital, via Manzoni 56, 20089 Rozzano, Milan, Italy
E-mail: luca.di_tommaso@hunimed.eu

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Renne SL, Sarcognato S, Sacchi D, et al. Hepatocellular carcinoma: a clinical and pathological overview. Pathologica 2021;113:203-217. https://doi.org/10.32074/1591-951X-295

Clinical background

Epidemiology. HCC incidence rates have been rising in the past 3 decades and similar trends are expected through 2030 [1]. The WHO stated in 2015 that HCC was the fifth most common cancer worldwide and the second most common cause of cancer-related death [2]. The global observatory on cancer reported that 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 [3]. By 2025, > 1 million individuals will be affected by liver cancer annually [3]. Over 90% of HCC cases occur in the setting of chronic liver disease. Cirrhosis from any etiology is the strongest risk factor for HCC [4,5]. 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.[2] The lifetime risk of these persons to develop cirrhosis and/or HCC is 15% to 40% [6,7]. 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 [8]. HCV infection affects 71 million persons all over the world.[2] 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 [5,9]. Nonetheless, HCV chronic infection is a major contributing factor to liver cancers in the USA, and is associated with 50% of cases.[10] NAFLD/NASH has a global prevalence of 24%, with the highest rates reported in South America and the Middle East (≈30-35%) [11]. It has been estimated that up to 25% of NAFLD can progress to NASH and that up to 20% of pa-

tients with NASH have cirrhosis [12]. NASH-associated cirrhosis carried a 2.4% to 12.8% increased risk of HCC [13]. Several studies have demonstrated that 25-30% of NASH-associated HCC occur in the absence of cirrhosis [14,15]. Since 2010, the proportion of HCC attributed to NASH has rapidly increased, currently representing 15-20% of cases in the West [16].

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 [17]. 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 [4,5]. 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 of 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) [18]. 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) [4,5]. 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% [4,5]. 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 [19]. The recurrence of HCC after hepatic resection rates as high as 70% at 5 years, even in patients with a single tumor ≤ 2 cm [20]. 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 [21]. 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 [22]. 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 [23]. 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 [24,25]. 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 [26]. 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 [23,27], with an estimated average of median overall survival of ~30 months [28]. Transarterial radioembolization (TARE) has shown efficacy in phase II investigations but has not been established as a primary standard of care by guidelines [29]. 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 [30]. In 2018 a phase II study demonstrated the efficacy of lenvatinib, which was then approved for advanced-stage HCC in the first-line setting [31]. In case of progression

to single-agent regimens, regorafenib [32], cabozantinib [33], and ramucirumab [34] 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) [35].

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) [36]. Single HCC with nodular morphology have favorable outcomes compared with those with multinodular, perinodular or infiltrative growth patterns [37-40]. 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 [41,42]. 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 [41,43]. At the molecular level, HG-DN, eHCC and pHCC are characterized by a progressive increase of TERT promoter mutation as a unique fingerprint [42]. 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 [4,5]. 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, macrotrabecular, 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 [44].

Studies based on high-throughput sequencing led to the identification of several molecular HCC subclasses [42, 45-51]. Regardless of the nomenclature used, HCC can be divided into 2 major subgroups (Fig. 1). The non-proliferation class [51-54] is 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 [55]. 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 [52,54,56], and the interferon subclass which presents a highly activated IL6-

Table I. Summary of the main pathological features of distinction between HGDN *vs* eHCC.

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	-	±	

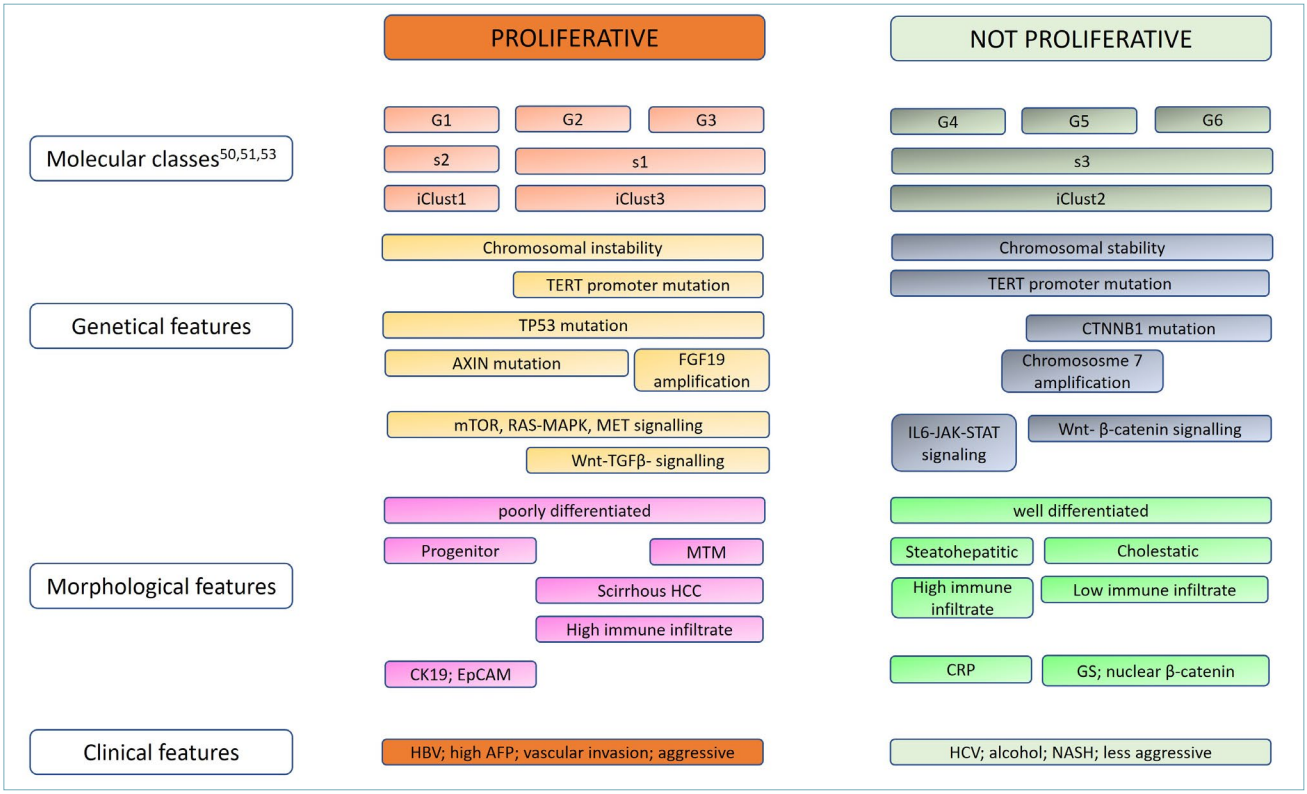

Figure 1. Classification of HCC. This scheme illustrates the correlations existing among molecular classes of HCC [50,51,53] and genetic, morphological and clinical features.

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 [45,50,51]. These HCCs are more aggressive and show poor histological differentiation, high vascular invasion and increased levels of AFP [55]. The proliferation-class can be further divided into two subclasses. The former, i.e. S1 or iCluster 3 [53,54], shows Wnt-TGFβ activation and immune-exhausted phenotype [56] and is barely recognizable at morphology; the latter, S2 or iCluster 1 [53,54], 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 [55].

According to the last WHO classification of liver tumors, about 1/3 of all HCC can be classified into specific subtypes: steatohepatitic, clear cell, macrotrabecolar massive, scirrhous, chromophobe, fibrolamellar, neutrophil- and lymphocyte- rich [44]. 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 [55-59]. CTNNB1 encodes β-catenin, a key intracellular transducer of the Wnt signaling pathway that regulates liver physiology and zonation [60]. 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 [61]. 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 [57]. One of these transporters, SLCO1B3, is responsible for the uptake of the MRI contrast agent gadoxetic acid [62].

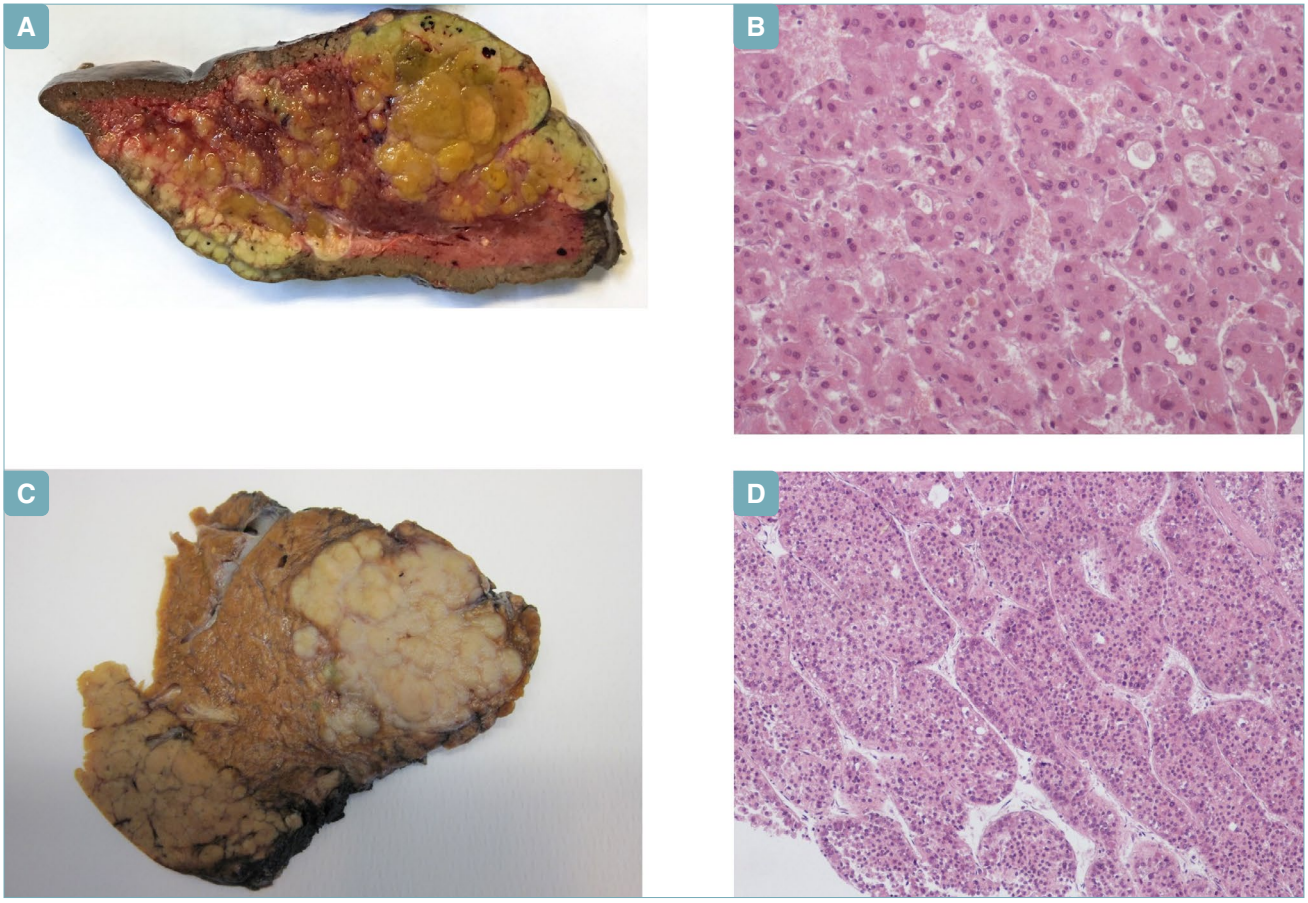

Figure 2. Pathology of HCC. This figure illustrates some of the most typical pathological features of HCC subtypes. (A, B) CTNNB1-mutated. In this fresh specimen, HCC presents as a mass made by several cluster of small and confluent green nodules (cd. "Multinodular confluent HCC") with some satellites (A); at histology the lesion is characterized by the presence of several pseudo-glandular structure filled by green material consistent with cholestasis (B). (C, D) Macrotrabecular Massive. In this fixed specimen the multiple confluent HCC nodules bulge over the surrounding flat fibrous strands (C); at microscopic level the lesion is characterized by a macrotrabecular pattern of growth (D).

Macro-trabecular-massive HCC (MTM-) HCC (Fig. 2C, D)

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 [57,63]. 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 [55,57]. Angiopoietin 2 is responsible for the destabilization of established blood vessels and subsequent vascular sprouting [64]. It also disrupts interactions between endothelial and peri-endothelial cells, which results in an increased sensitivity to VEGFA.

Scirrhous 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 [65]. 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 [55 65].

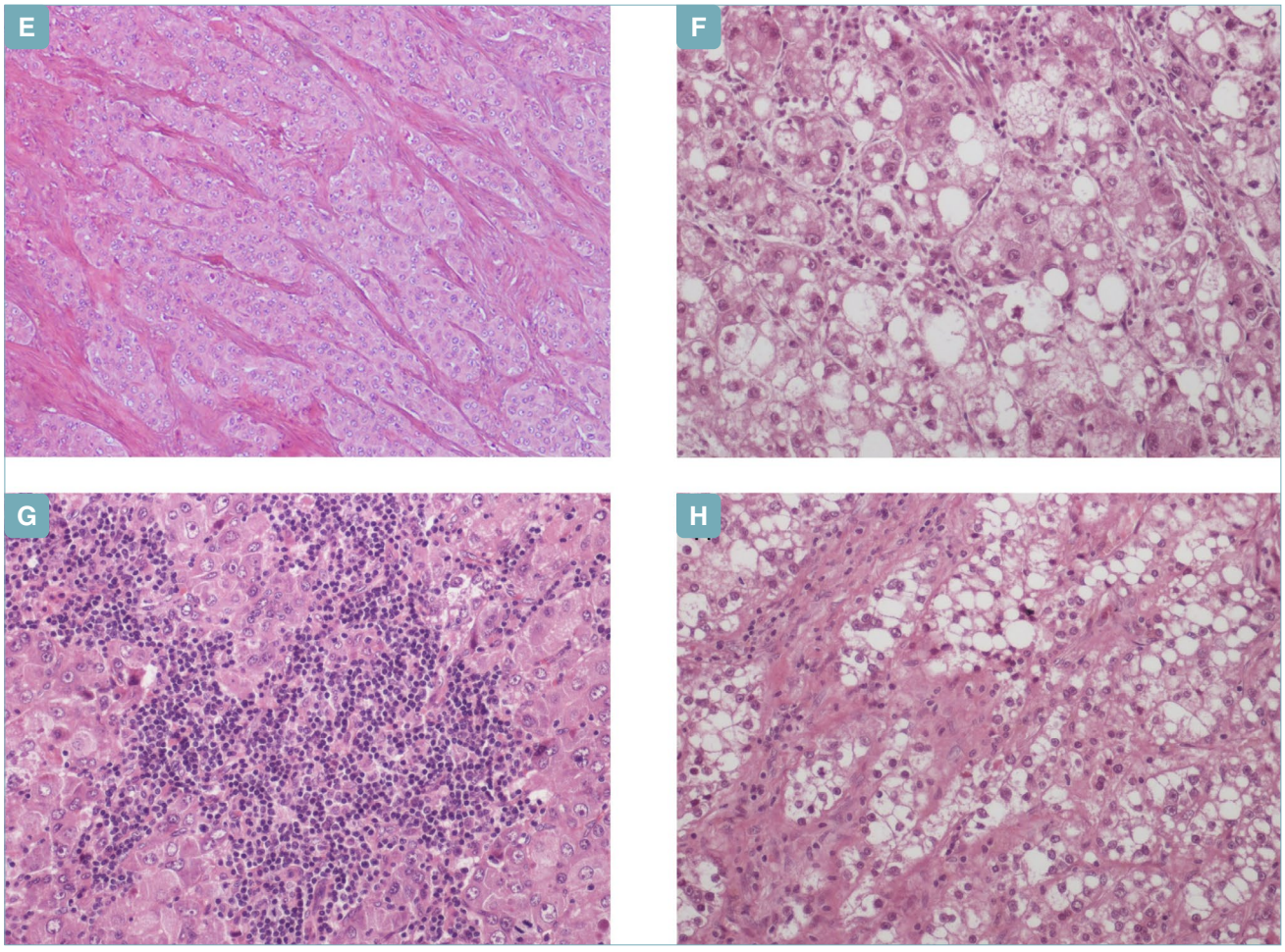

Figure 2. Pathology of HCC. (E) Scirrhous. This HCC is characterized by abundant stroma separating neoplastic trabeculae. (F) In this HCC, neoplastic cells are characterized by diffuse steatosis and occasional cell ballooning; the tumor shows scattered inflammatory infiltrates. (G) Lymphocyte-rich. A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

Steatohepatitic HCC (Fig. 2F)

This subtype is characterized by inflammatory infiltrates, cell ballooning, peri-cellular fibrosis and Mallory-Denk bodies [66]. At phenotypical level neoplastic cells show overexpression of C-reactive protein (CRP) - a target gene of JAK/STAT signaling [55]. 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 [44]. 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 [67]. 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 [67,68]. As opposite to other settings, such as colon and lung cancer, where lymphocytic infiltration has been linked with microsatellite instability and/or high mutational [69], none of these HCCs were microsatellite instable or associated with a higher number of somatic mutations [56,67].

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 [44,70].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 [70] later accepted by the WHO [44]. 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 [44].
A recent study showed that HCC and CCA components had very similar global gene expression profiles, thus suggesting a monoclonal origin [71]. 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 [71].

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 [57,63], but not validated in external cohort [59]. By contrast, a robust prognostic significance has been proposed [72] and later validated [59] for a peculiar vascular phenotype, characterized by CD34+ vessels encapsulating tumor cluster (VETC). VETC might be present in up to 40% of HCC [59] and associates with higher attitude of tumoral cells to infiltrate vessels. Interestingly, a recent study demonstrated that this phenotype predicts the response to sorafenib [73].

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

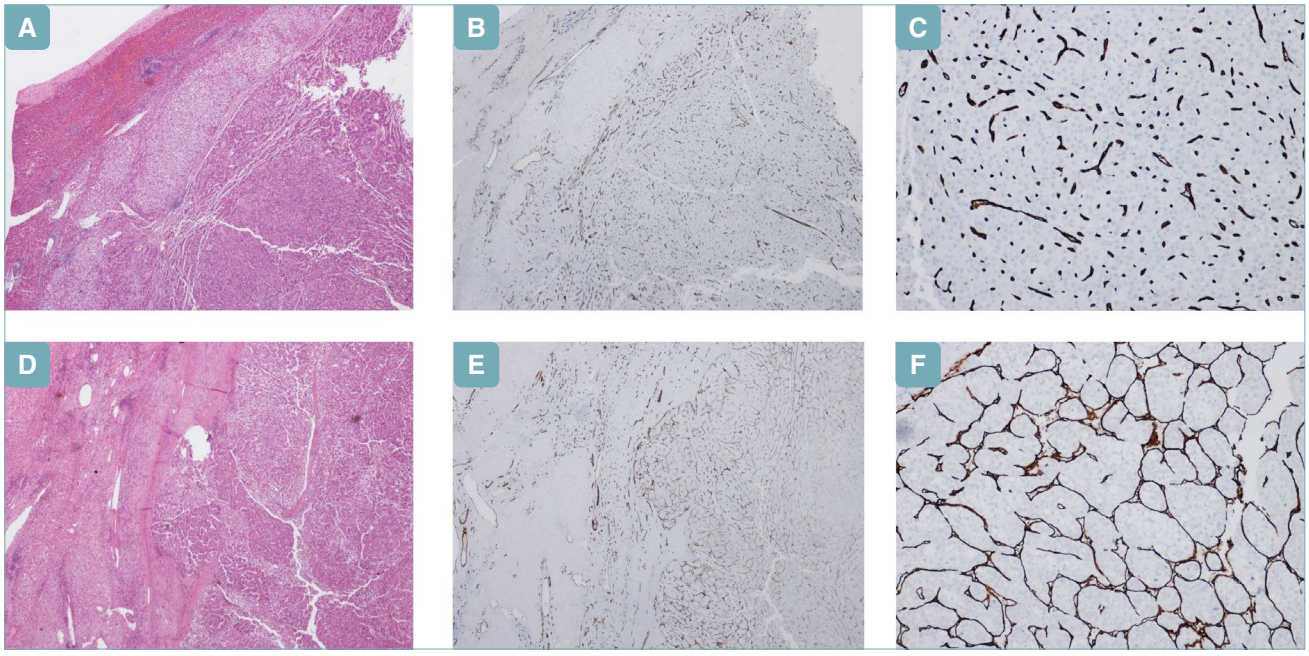

Figure 3. Vascularization of HCC. (A, B, C) The images illustrate an HCC (lower right corner, A) with a rich vascular support as highlighted by a CD34 staining (B); at higher magnification CD34+ vessels show a capillary distribution; (C). (D, E, F) also this HCC (right part, D) shows a rich vascular network when stained with CD34 (E); in this cases however, CD34+ vessels encapsulate clusters of neoplastic cells (F) featuring a peculiar phenotype described as VETC [59,72].

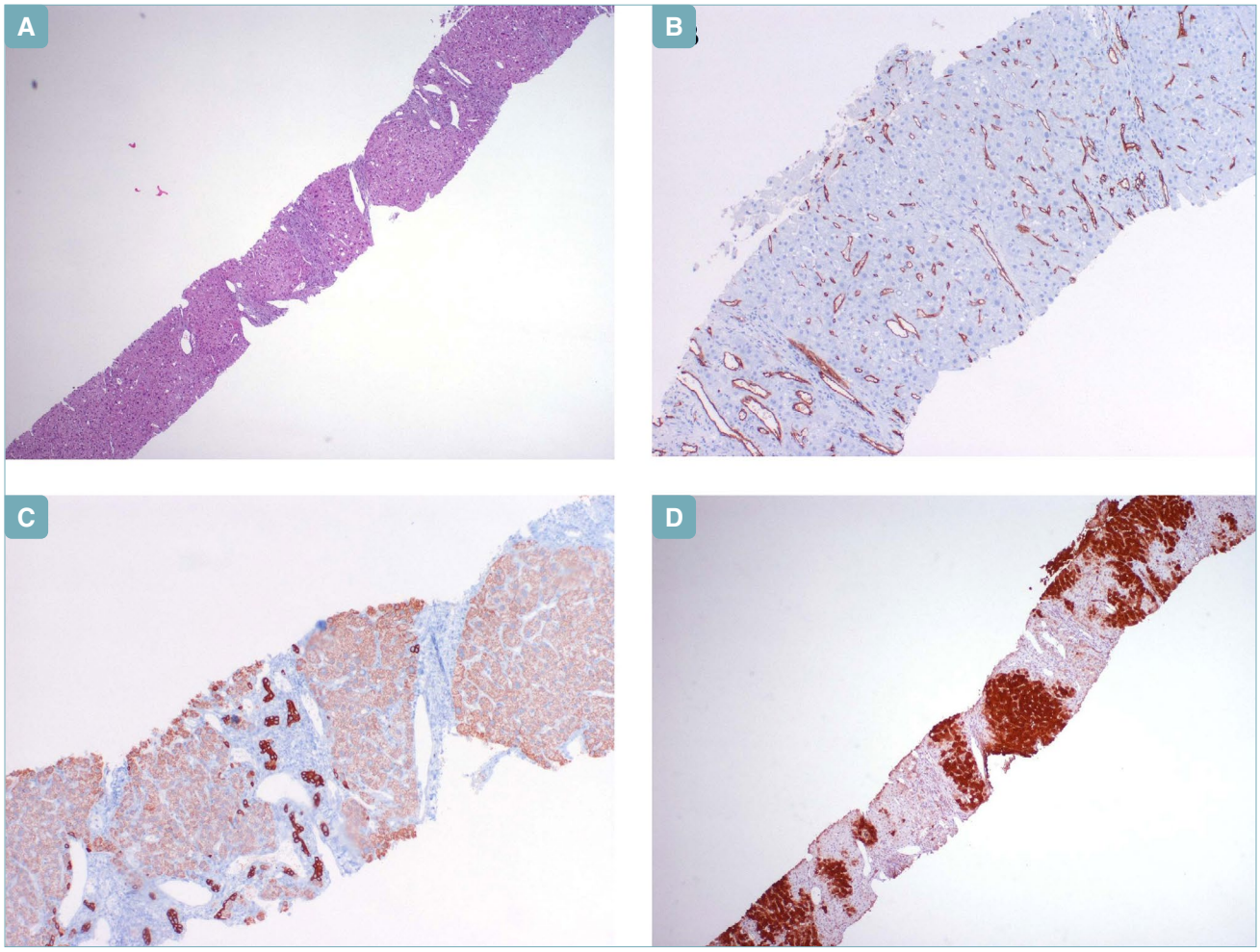

Figure 4. Diagnostic of well differentiated hepatocellular lesions in healthy liver: Focal Nodular Hyperplasia. (A) This liver biopsy illustrates an area characterized by the presence of fibrous septa: a feature which might raise the suspect of a not adequate specimen; (B) in such cases the expression of CD34 by the endothelium of sinusoids can be of great help: indeed, a diffuse increase is in keeping with lesional sampling; (C) CK7 highlights that the lesion is characterized by the presence of portal tracts; (D) GS immunostaing proves a pathognomonic strong, map-like, staining for GS. The mopho-phenoptypical findings are conclusive for an FNH.

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 $GS^{+map\text{-}like}/SAA^{-}/CRP^{-}$ supports FNH while GS-/SAA+/CRP+ or $GS^{+non\text{-}map\text{-}like}/SAA^{+}/PCR^{+}$ 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 Fatty Acid Binding protein (LFABP) in tumoral hepatocytes will prove it. A non-steatotic and non-atypical adenoma with a LFABP-/SAA-/CRP-/GS- phenotype should be classified as u-HA. Finally, when the lesion lacks pseudo-portal tracts, inflammatory/teleangectatic morphol-

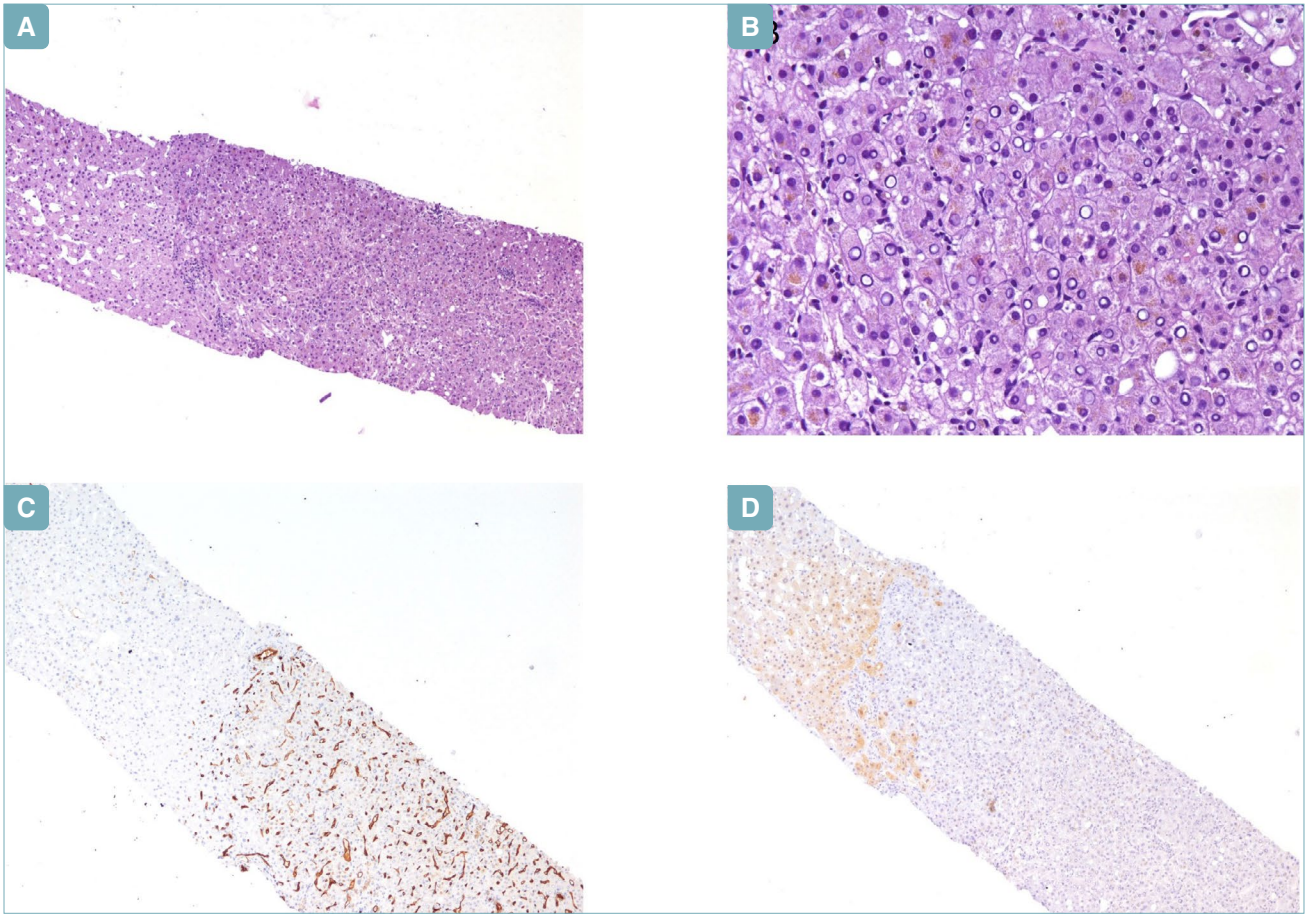

Figure 5. Diagnosis of well differentiated hepatocellular lesions in healthy liver: Steatotic Hepatocellular Adenoma. (A) This liver biopsy documents a well differentiated lesion which, at H/E, is barely detectable (right part); (B) at higher magnification lesional hepatocytes are characterized by clear nuclei; (C) CD34 staining highlights the lesion; (D) lesional hepatocytes, as compared to the surrounding, do not stain for LFABP. The morpho-phenotypical findings are conclusive for a steatotic HA.

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 [41]. 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 periseptal 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-

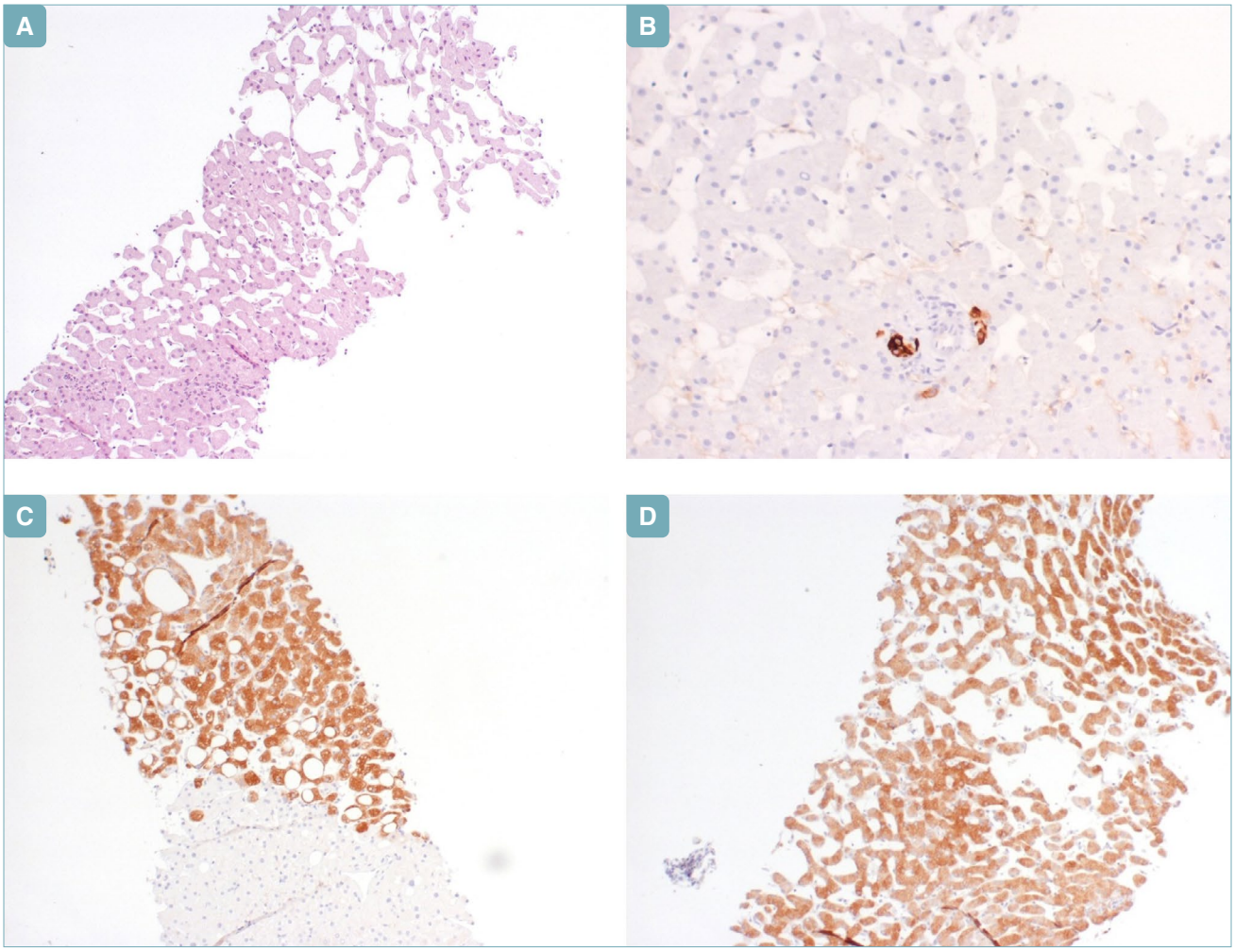

Figure 6. Diagnosis of well differentiated hepatocellular lesions in healthy liver: teleangiectatic/inflammatory Hepatocellular Adenoma. (A) This liver biopsy illustrates a well differentiated hepatocellular lesion characterized, at higher magnification, by teleangiectatic vessels; (B) CK7 highlights that the lesion is characterized by the presence of scattered pseudo-portal tracts; (C and D) the lesion is immunoreactive for SAA and PCR. The morpho-phenotypical findings are conclusive for telenagiectatic HA.

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

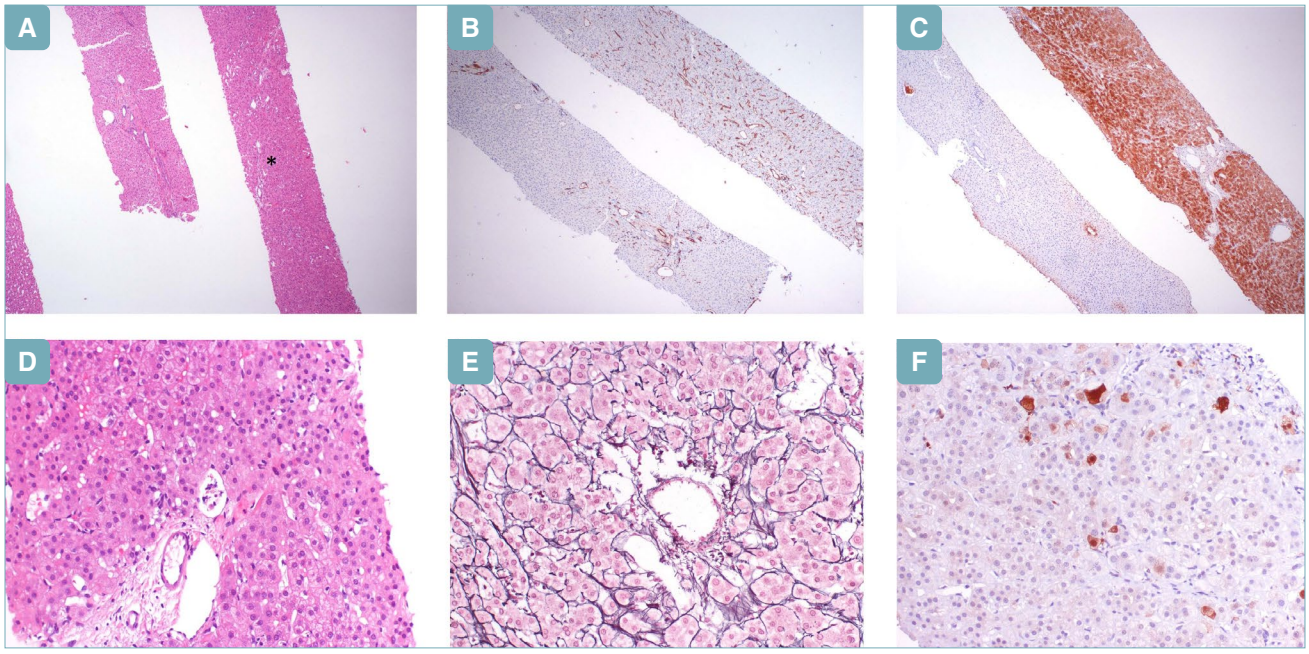

Figure 7. Diagnosis of well differentiated hepatocellular lesions in healthy liver: Atypical Hepatocellular Adenoma. (A) These biopsies document a well differentiated hepatocellular lesion (*) and the surrounding parenchyma; (B) the lesion, which is barely seen on H/E at scanning magnification, is highlighted 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%

of CK19. The correct definition of the histotype enriches the pathological report with prognostic information. The MTM histotype, has a poorer outcome [57,63] while the lymphocyte-rich HCC has a better prognosis [44]. 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 [79] 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 [56,80]. HCC grading systems (Edmondson-Steiner and WHO, with several "home-made" variations) strongly predict patient outcome in liver resection or transplantation [81], with the worst grade driving HCC prognosis [82]. 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 [83-85]. MVI occurs at the rates of 25%,

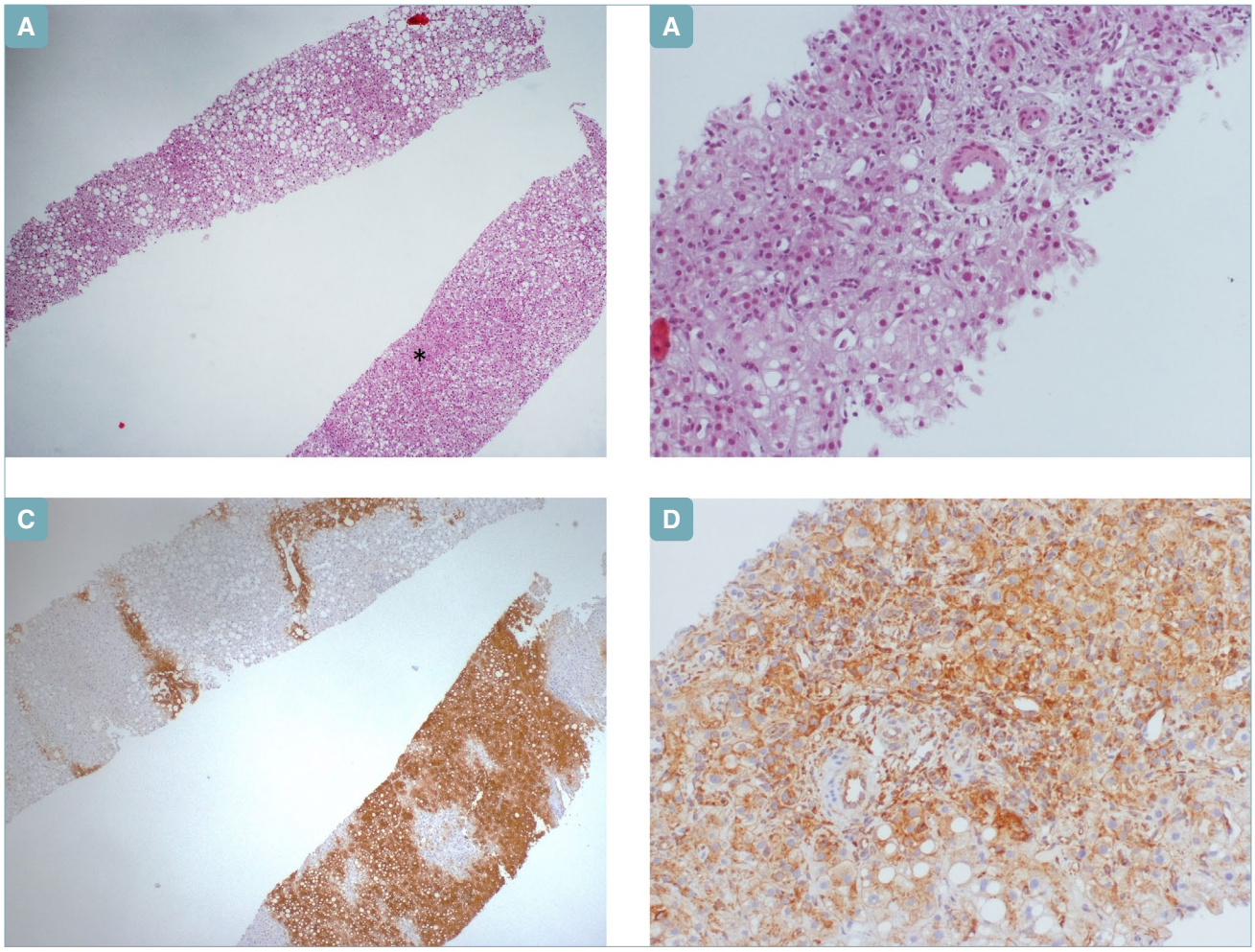

Figure 8. Diagnosis of well differentiated hepatocellular lesions in hepatitis/cirrhotic liver. (A) This biopsy document, in the setting of a chronic steatohepatitic background, the presence of a well differentiated lesion (*); (B) at higher magnification the lesion is characterized by unpaired arteries and pseudo-glandular arrangement of hepatocytes; (C) the nodule showed an diffuse and intense immunoreactivity for GS (non neoplastic parenchyma showed a laminar staining); (D) neoplastic cells are also immunoreactive for CHC. The morpho-phenotypical findings are conclusive for an eHCC.

40%, 55% and 63% in HCC < 3, 3-5, 5-6.5, and > 6.5 cm [86]. Accordingly immunohistochemical markers surrogate of MVI are intensively investigated [87,88]. 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 [89].

References

1 Petrick JL, Kelly SP, Altekruse SF, et al. Future of hepatocellular carcinoma incidence in the united states forecast through 2030. J Clin Oncol 2016;34:1787-1794. https://doi.org/10.1200/JCO.2015.64.7412

2 World Health Organization. Global Hepatitis Report 2017. Geneva, Switzerland: World Health Organization 2017.

3 International Agency for Research on Cancer. GLOBOCAN 2018. IARC https://gco.iarc.fr/today/online-analysis-map?v=2020&mode=population&mode_population=continents&population=900&populations=900&key=asr&sex=0&cancer=11&type=0&statistic=5&prevalence=0&population_groupearth&color_palette=default&map_scale= quantile&map_nb_colors=5&continent=0&rotate=%255B10%252C0%255D (2020)

4 European Association for the Study of the Liver. EASL clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2018;69:182-236. https://doi.org/10.1016/j.jhep.2018.03.019

5 Marrero JA, Kulik LM, Sirlin CB, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. Hepatology 2018;68:723-750. https://doi.org/10.1002/hep.29913.PMID: 29624699

6 Fattovich G, Stroffolini T, Zagni I, et al. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004;127:S35-S50(5 suppl 1). https://doi.org/10.1053/j.gastro.2004.09.014

7 Lok AS. Chronic hepatitis B. N Engl J Med 2002;346:1682-1683. https://doi.org/10.1056/NEJM200205303462202

8 Akinyemiju T, Abera S, Ahmed M, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level. JAMA Oncol 201;3:1683. https://doi.org/10.1001/jamaoncol.2017.3055

9 Kanwal F, Kramer J, Asch SM, et al. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. Gastroenterology 2017;153:996-1005.e1. https://doi.org/10.1053/j.gastro.2017.06.012

10 Perz JF, Armstrong GL, Farrington LA, et al. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol 2006;45:529-538. https://doi.org/10.1016/j.jhep.2006.05.013

11 Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2018;15:11-20. https://doi.org/10.1038/nrgastro.2017.109

12 Khan FZ, Perumpail RB, Wong RJ, et al. Advances in hepatocellular carcinoma: nonalcoholic steatohepatitis-related hepatocellular carcinoma. World J Hepatol 2015;7:2155-2161. https://doi.org/10.4254/wjh.v7.i18.2155

13 White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. Clin Gastroenterol Hepatol 2012;10:1342-1359.e2. https://doi.org/10.1016/j.cgh.2012.10.001

14 Kawada N, Imanaka K, Kawaguchi T, et al. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. J Gastroenterol 2009;44:1190-1194. https://doi.org/10.1007/s00535-009-0112-0

15 Paradis V, Zalinski S, Chelbi E, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. Hepatology 2009;49:851-859. https://doi.org/10.1002/hep.22734

16 Estes C, Razavi H, Loomba R, et al. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology 2018;67:123-133. https://doi.org/10.1002/hep.29466

17 van der Pol CB, Lim CS, Sirlin CB, et al et al. Accuracy of the liver imaging reporting and data system in computed tomography and magnetic resonance image analysis of hepatocellular carcinoma or overall malignancy - a systematic review. Gastroenterology 2019;156:976-986. https://doi.org/10.1053/j.gastro.2018.11.020

18 Mitchell DG, Bruix J, Sherman M, et al. LI-RADS (Liver Imaging Reporting and Data System): summary, discussion, and consensus of the LI-RADS Management Working Group and future directions. Hepatology 2015;61:1056-1065. https://doi.org/10.1002/hep.27304

19 Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med1996;334:693-700. https://doi.org/10.1056/NEJM199603143341104

20 Roayaie S, Obeidat K, Sposito C, et al. Resection of hepatocellular cancer ≤2 cm: results from two Western centers. Hepatology 2013;57:1426-1435. https://doi.org/10.1002/hep.25832

21 Imamura H, Matsuyama Y, Tanaka E, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol 2003;38:200-207. https://doi.org/10.1016/s0168-8278(02)00360-4

22 Tabrizian, P. et al. A US multicenter analysis of 2529 HCC patients undergoing liver transplantation: 10-year outcome assessing the role of down-staging to within Milan criteria [abstract 15]. Hepatology 2019;70:10-11.

23 Heimbach JK, Kulik LM, Finn RS, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology 2018;67,:358-380. https://doi.org/10.1002/hep.29086

24 Germani G, Pleguezuelo M, Gurusamy K, et al. Clinical outcomes of radiof- requency ablation, percutaneous alcohol and acetic acid injection for hepatocelullar carcinoma: a meta-analysis. J Hepatol 2010;52:380-388. https://doi.org/10.1016/j.jhep.2009.12.004

25 Cho YK, Kim JK, Kim MY, et al. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. Hepatology 2009;49:453-459. https://doi.org/10.1002/hep.22648

26 Llovet, J. & Bruix, J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. Hepatology 2003;37:429-442. https://doi.org/10.1053/jhep.2003.50047

27 Vogel A, Cervantes A, Chau I, et al. Hepatocellular carcinoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018;29 (Suppl. 4), iv238-iv255. https://doi.org/10.1093/annonc/mdy308

28 Llovet, JM, de Baere T, Kulik L, et al. Locoregional therapies in the era of molecular and immune treatments for hepatocellular carcinoma. Nat. Rev. Gastroenterol. Hepatol 2020;https://doi.org/10.1038/s41575-020-00395-0

29 Salem R, Gordon AC, Mouli S, et al. Y90 radioembolization significantly prolongs time to progression compared with chemoembolization in patients with hepatocellular carcinoma. Gastroenterology 2016;151:1155-1163.e2. https://doi.org/10.1053/j.gastro.2016.08.029

30 Llovet, JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-390. https://doi.org/10.1056/NEJMoa0708857

31 Kudo M, Finn RS, Qin S, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet 2018;391:1163-1173. https://doi.org/10.1016/S0140-6736(18)30207-1

32 Bruix J, Qin S, Merle P, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017;389:56-66. https://doi.org/10.1016/S0140-6736(16)32453-9

33 Abou-Alfa GK, Meyer T, Cheng AL, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. N Engl J Med 2018;379:54-63. https://doi.org/10.1056/NEJMoa1717002

34 Zhu AX, Kang YO, Yen CJ, et al. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial.

Lancet Oncol 2019;20:282-296. https://doi.org/10.1016/S1470-2045(18)30937-9

35 Finn RS, Qin S, Ikeda M, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med2020;382:1894-1905. https://doi.org/10.1056/NEJMoa1915745

36 Jang JY, Lee JS, Kim HJ, et al. The General Rules for the Study of Primary Liver Cancer. Journal of Liver Cancer 2017;17:19-44. https://doi.org/10.17998/jlc.17.1.19

37 Hui AM, Takayama T, Sano K, et al. Predictive value of gross classification of hepatocellular carcinoma on recurrence and survival after hepatectomy. J Hepatol 2000;33:975-979. https://doi.org/10.1016/s0168-8278(00)80131-2

38 Shimada M, Rikimaru T, Hamatsu T, et al. The role of macroscopic classification in nodular-type hepatocellular carcinoma. Am J Surg 2001;182:177-182. https://doi.org/10.1016/s0002-9610(01)00682-1

39 Tsujita E, Yamashita Y, Takeishi K, et al. The clinicopathological impact of gross classification on solitary small hepatocellular carcinoma. Hepatogastroenterology 2013;60:1726-1730

40 Lee Y, Park H, Lee H, et al. The Clinicopathological and Prognostic Significance of the Gross Classification of Hepatocellular Carcinoma. J Pathol Transl Med 2018;52:85-92. https://doi.org/10.4132/jptm.2017.11.13

41 International Consensus Group for Hepatocellular Neoplasia & The International Consensus Group for Hepatocellular N. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology 2009;49:658-664. https://doi.org/10.1002/hep.22709

42 Nault JC, Mallet M, Pilati C, et al. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. Nat Commun 2013;4:2218. https://doi.org/10.1038/ncomms3218

43 Di Tommaso L, Sangiovanni A, Borzio M, et la. Advanced precancerous lesions in the liver. Best Pract Res Clin Gastroenterol 2013;27:269-284. https://doi.org/10.1016/j.bpg.2013.03.015

44 WHO classification of Tumours Editorial Board. Digestive system tumours. 5th ed, vol. 1. Lyon: International Agency for Research on cancern 2019.

45 Zucman-Rossi J, Villanueva A, Nault JC, et al. Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology 2015;149;1226-1239e1224. https://doi.org/10.1053/j.gastro.2015.05.061

46 Ahn SM, Jang SJ, Shim JH, et al. A genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. Hepatology 2014;60:1972-1982. https://doi.org/10.1002/hep.27198

47 Schulze K, Imbeaud S, Letouze E, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet 2015;47:505-511. https://doi.org/10.1038/ng.3252

48 Guichard C, Amaddeo G, Imbeaud S, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012;44:694-698. https://doi.org/10.1038/ng.2256

49 Totoki Y, Tatsuno K, Covington K, et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. Nat Genet 2014;46:1267-1273. Epub 2014 Nov 2. https://doi.org/10.1038/ng.3126

50 Hoshida Y, Nijman SM, Kobayashi M, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res 2009;69:7385-7392. https://doi.org/10.1158/0008-5472.CAN-09-1089

51 Boyault S, Rickman DS, de Reynies A, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 2007;45:42-52. https://doi.org/10.1002/hep.21467

52 Chiang DY, Villanueva A, Hoshida Y, et al. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. Cancer Res 2008;68:6779-6788. https://doi.org/10.1158/0008-5472.CAN-08-0742

53 Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell 2017;169:1327-1341.e23. https://doi.org/10.1016/j.cell.2017.05.046

54 Lee JS, Heo J, Libbrecht L, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat. Med 2006;12:410-416. https://doi.org/10.1038/nm1377

55 Calderaro J, Ziol M, Paradis V, et al. Molecular and histological correlations in liver cancer. J. Hepatol 2019;71:616-630. https://doi.org/10.1016/j.jhep.2019.06.001

56 Sia D, Jiao Y, Martinez-Quetglas I, et al. Identification of an immune-specific class of hepatocellular carcinoma, based on molecular features. Gastroenterology 2017;153:812-826. https://doi.org/10.1053/j.gastro.2017.06.007

57 Calderaro J, Couchy G, Imbeaud S, et al. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. J Hepatol 2017;67:727-738. https://doi.org/10.1016/j.jhep.2017.05.014

58 Audard V, Grimber G, Elie C, et al. Cholestasis is a marker for hepatocellular carcinomas displaying beta-catenin mutations. J Pathol 2007;212:345-352. https://doi.org/10.1002/path.2169

59 Renne SL, Woo HY, Allegra S, et al. Vessels Encapsulating Tumor Clusters (VETC) Is a Powerful Predictor of Aggressive Hepatocellular Carcinoma. Hepatology 2020;71:183-195. https://doi.org/10.1002/hep.30814

60 Monga S.P. Beta-catenin signaling and roles in liver homeostasis, injury, and tumorigenesis. Gastroenterology 2015;148:1294-1310. https://doi.org/10.1053/j.gastro.2015.02.056

61 Rebouissou S, Franconi A, Calderaro J, et al. Genotype-phenotype correlation of CTNNB1 mutations reveals different ß-catenin activity associated with liver tumor progression. Hepatology 2016;64:2047-2061. https://doi.org/10.1002/hep.28638

62 Ueno A, Masugi Y, Yamazaki K, et al. OATP1B3 expression is strongly associated with Wnt/beta-catenin signalling and represents the transporter of gadoxetic acid in hepatocellular carcinoma. J Hepatol 2014;61:1080-1087. https://doi.org/10.1016/j.jhep.2014.06.008

63 Ziol M, Pote N, Amaddeo G, et al. Macrotrabecular-massive hepatocellular carcinoma: a distinctive histological subtype with clinical relevance. Hepatology 2018;68:103-112. https://doi.org/10.1002/hep.29762

64 Gerald D, Chintharlapalli S, Augustin H, et al. Angiopoietin-2: an attractive target for improved antiangiogenic tumor therapy. Cancer Res 2013;73:1649-1657. https://doi.org/10.1158/0008-5472.CAN-12-4697

65 Seok JY, Na DC, Woo HG, et al. A fibrous stromal component in hepatocellular carcinoma reveals a cholangiocarcinoma-like gene expression trait and epithelial-mesenchymal transition. Hepatology 2012;55:1776-1786. https://doi.org/10.1002/hep.25570

66 Salomao M, Yu WM, Brown RB, et al. Steatohepatitic hepatocellular carcinoma (SH-HCC): a distinctive histological variant of HCC in hepatitis C virus-related cirrhosis with associated

NAFLD/NASH. Am J Surg Pathol 2010;34:1630-1636. https://doi.org/10.1097/PAS.0b013e3181f31caa

67 Chan AW, Tong JH, Pan Y, et al. Lymphoepithelioma-like hepatocellular carcinoma: an uncommon variant of hepatocellular carcinoma with favorable outcome. Am J Surg Pathol 2015;39:304-312. https://doi.org/10.1097/PAS.0000000000000376

68 Calderaro J, Rousseau B, Amaddeo G, et al. Programmed death ligand 1 expression in hepatocellular carcinoma: relationship with clinical and pathological features. Hepatology 2016;64:2038-2046. https://doi.org/10.1002/hep.28710

69 Turajlic S, Litchfield K, Xu H, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. Lancet Oncol 2017;18:1009-1021. https://doi.org/10.1016/S1470-2045(17)30516-8

70 Brunt E, Aishima S, Clavien P-A, et al. cHCC-CCA: consensus terminology for primary liver carcinomas with both hepatocytic and cholangiocytic differentation. Hepatology 2018;68:113-126. https://doi.org/10.1002/hep.29789

71 Xue R, Chen L, Zhang C, et al. Genomic and transcriptomic profiling of combined hepatocellular and intrahepatic cholangiocarcinoma reveals distinct molecular subtypes. Cancer Cell 2019;35. https://doi.org/10.1016/j.ccell.2019.04.007.932-947.e8

72 Fang JH, Zhou HC, Zhang C, et al. A novel vascular pattern promotes metastasis of hepatocellular carcinoma in an epithelial-mesenchymal transition-independent manner. Hepatology 2015;62:452-65. https://doi.org/10.1002/hep.27760

73 Fang JH, Xu L, Shang LR, et al. Vessels That Encapsulate Tumor Clusters (VETC) Pattern Is a Predictor of Sorafenib Benefit in Patients with Hepatocellular Carcinoma. Hepatology 2019;70:824-839. https://doi.org/10.1002/hep.30366

74 Di Tommaso L, Franchi G, Park YN, et al. Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. Hepatology 2007;45:725-734 https:://doi.org/10.1002/hep.21531

75 Di Tommaso L, Destro A, Seok JY, et al. The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. Journal of Hepatology 2009;50:746-754. https://doi.org/10.1016/j.jhep.2008.11.014

76 Di Tommaso L, Destro A, Fabbris V, et al. Diagnostic accuracy of clathrin heavy chain staining in a marker panel for the diagnosis of small hepatocellular carcinoma. Hepatology 2011;53:1549-1557. https://doi.org/10.1002/hep.24218

77 Sempoux C, Gouw ASH, Dunet V, et al. Predictive Patterns of Glutamine Synthetase Immunohistochemical Staining in CTNNB1-mutated Hepatocellular Adenomas. Am J Surg Pathol 2021;45:477-487. https://doi.org/10.1097/PAS.0000000000001675

78 Di Tommaso L, Roncalli M. Tissue biomarkers in hepatocellular tumors: which, when, and how. Front Med (Lausanne) 2017;23;4:10. https://doi.org/10.3389/fmed.2017.00010

79 Kurebayashi Y, Ojima H, Tsujikawa H, et al. Landscape of immune microenvironment in hepatocellular carcinoma and its additional impact on histological and molecular classification. Hepatology 2018;68:1025-1041. Epub 2018 Jul 25. https://doi.org/10.1002/hep.29904.

80 Pinyol R, Sia D, Llovet JM. Immune Exclusion-Wnt/CTNNB1 Class Predicts Resistance to Immunotherapies in HCC. Clin Cancer Res 2019;25:2021-23. https://doi.org/10.1158/1078-0432.CCR-18-3778

81 Martins-Filho SN, Paiva C, Azevedo RS, et al. Histological Grading of Hepatocellular Carcinoma-A Systematic Review of Literature. Front Med (Lausanne) 2017;4:193. https://doi.org/10.3389/fmed.2017.00193. eCollection 2017

82 Han DH, Choi GH, Kim KS, et al. Prognostic significance of the worst grade in hepatocellular carcinoma with heterogeneous histologic grades of differentiation. J Gastroenterol Hepatol 2013;28:1384-1390. https://doi.org/10.1111/jgh.12200

83 Lauwers GY, Terris B, Balis UJ, et al. International Cooperative Study Group on Hepatocellular Carcinoma. Prognostic histologic indicators of curatively resected hepatocellular carcinomas: a multi-institutional analysis of 425 patients with definition of a histologic prognostic index. Am J Surg Pathol 2002;26:25-34. https://doi.org/10.1097/00000478-200201000-00003

84 Qin LX, Tang ZY. The prognostic significance of clinical and pathological features in hepatocellular carcinoma. World J Gastroenterol 2002;8:193-199. https://doi.org/10.3748/wjg.v8.i2.193

85 Rodríguez-Perálvarez M, Luong TV, Andreana L, et al. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. Ann Surg Oncol 2013;20:325-339. https://doi.org/10.1245/s10434-012-2513-1

86 Pawlik TM, Delman KA, Vauthey JN, et al. Tumor size predicts vascular invasion and histologic grade: Implications for selection of surgical treatment for hepatocellular carcinoma. Liver Transpl 2005;11:1086-1092. https://doi.org/10.1002/lt.20472

87 Poté N, Cauchy F, Albuquerque M, et al. Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion. J Hepatol 2015;62:848-854. https://doi.org/10.1016/j.jhep.2014.11.005

88 Poté N, Cauchy F, Albuquerque M, et al. Contribution of virtual biopsy to the screening of microvascular invasion in hepatocellular carcinoma: a pilot study. Liver Int 2018;38:687-694. https://doi.org/10.1111/liv.13585

89 Kim H, Choi GH, Na DC, et al. Human hepatocellular carcinomas with "Stemness"- related marker expression: keratin 19 expression and a poor prognosis. Hepatology 2011;54:1707-17017. https://doi.org/10.1002/hep.24559