MRI and pathology aspects of hypervascular nodules in cirrhotic liver: from dysplasia to hepatocarcinoma

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Abstract
The incidence of hepatocellular carcinoma (HCC) has been constantly increasing over the last years mainly due to hepatitis C infection and cirrhosis. The new developments in imaging technology, including magnetic resonance imaging (MRI) and computed tomography (CT), allow a better diagnosis of HCC. Cirrhosis is characterized by formation of nodules from regenerative nodules to dysplastic nodules, followed by HCC. Thus, the differential diagnosis of hypervascular hepatic lesions is important, especially in the nodules smaller than 2 cm, although their characterization may be difficult even when histopathology is used. A multistep approach with the comparison of clinical data, pathological findings and imaging features is useful for a more accurate diagnosis. MRI has the ability to assess the same lesions features as CT and to better characterize the enhancement patterns of nodules combined with the lack of irradiation. Moreover, new liver specific contrast agents and imaging techniques as diffusion-weighted (DWI) sequences are available. Regenerative and low-grade dysplastic nodules demonstrate contrast enhancement similar to that of surrounding liver parenchyma, compare to high-grade dysplastic nodules, which may show arterial enhancement similar to that seen in HCC. We present a review of the MR imaging and histopathological features of hypervascular nodules in the cirrhotic liver, with reference to the transition from dysplasia to HCC.

Keywords: MRI, regenerative nodules, dysplastic nodules, hepatocellular carcinoma.

Introduction
Hepatocellular carcinoma (HCC) is one of the most common tumor in the world and is the third most common cause of death by cancer, following lung and stomach cancer [1, 2].

Chronic hepatitis B and C are recognized as the major factors, which increase the risk of HCC [3]. Cirrhosis is another risk factor for HCC, with an annual risk of developing HCC between 1% and 6% [4].

The screening of high-risk groups of developing HCC is useful for detection of early stage tumors and for initiation of curative therapy [5]. The current screening tests used for patients with cirrhosis are alpha-fetoprotein (AFP) level testing and ultrasonography (US) with a sensitivity of 50–60% [6]. Magnetic resonance imaging (MRI) or computed tomography (CT) are the best imaging techniques currently available for the diagnosis of HCC, in the event of abnormal US and AFP [7].

Liver cirrhosis is characterized by a distorted hepatic architecture with bridging fibrosis and a spectrum of hepatocellular nodules [8]. Most are benign regenerative nodules; however, they may progress to become dysplastic nodules or HCCs [9]. Therefore, imaging evaluation of these hepatocellular nodules is important for an optimal management and evaluation.

Evaluation of liver nodules
Currently, there are several morphological evaluation criteria that together can differentiate between regenerative nodules, dysplastic nodules and HCC; these include macroscopic criteria (size, growth pattern, capsular invasion), microscopic (mitotic activity, presence of necrosis, vascular invasion, tumor heterogeneity and growth pattern), criteria based on immunohistochemistry including the quantification of the sinusoidal capillarization and tumor neoangiogenesis [10]. However, some of these criteria can be assessed accurately only on surgical resection specimens that are not always available and morphological study of lesions in the context of imaging analysis, is an important step for improving the prognosis and therapeutic management [11].

The transition from dysplastic nodules to HCC is a continuous process due to histopathological changes that nodules undergo which encompasses different MR features. A 1.5- or 3.0-T MRI system with an abdominal coil is used for liver evaluation in most medical establishments. The standard technique can include unenhanced T1, T2 and T2 gradient-weighting sequences with multiple pulse sequences and variable parameters.

The use of diffusion-weighted imaging (DWI) sequences with apparent diffusion coefficient (ADC) mapping are useful to evaluate hepatocellular nodules [12–14]. There are several studies that demonstrated the superiority of DWI over T2-weighted MR imaging for detection of liver lesions [15, 16]. The DWI sequence should always be concurrently interpreted with conventional MR imaging findings, with the main disadvantage of a lack of standardization [14].

The contrast agents used in enhanced acquisition are
gadolinium chelates with low molecular weight, hepatocellular agents, and superparamagnetic iron oxide (SPIO) particles. The most widely available contrast agents are represented by gadolinium-based extracellular contrast agents [17–21].

Information about tissue vascularity are provided by the first class of contrast agents, respectively the gadolinium chelates, using T1-weighted images acquired before and in multiple phases after the administration. The most used hepatocellular agents are gadobenate dimeglumine (Gd–BOPTA) and gadoteric acid disodium (Gd–EOB–DTPA) [22]. Other MR imaging contrast agents are SPIO agents. The use of dynamic and static T1 and T2 MR imaging sequences appears to provide clinically more useful patterns of enhancement [23–25].

Several studies showed the useful of double contrast-enhanced MR imaging in which both SPIO particles and a gadolinium chelate are administered in the same examination with increase in detection of hepatic tumors [26, 27].

Regenerative nodules

A permanent injury to the liver parenchyma is the trigger for the appearance of regenerative nodules, which are benign nodules surrounded by fibrous septa [28, 29].

For MR images, the signal intensity is variable in T1-weighted images and iso-/hypointense on T2-weighted images (Figure 1A). The signal of these lesions may be high in T1 sequences, when it contains a substantial amount of proteins or lipids (Figure 1B). Because of normal hepatocellularity and function of phagocytes, the regenerative nodules enhance similar or slightly less than surrounding parenchyma after contrast administration [30, 31]. A result of fat deposition is the appearance of steatotic nodules, which are usually plurifocal with high signal on in-phase gradient images and low signal intensity in out-of-phase sequences [31, 32]. Another type of regenerative lesions are siderotic nodules, which are the effect of iron deposition with hypointensity in both T1- and T2-weighted images [33].

The gross pathological appearance aspect of regenerative nodules usually indicates 1–3 cm in size, being round, prominent and well defined, green (bile impregnation) or yellow-brown (steatosis). Usually, these nodules are larger than typical cirrhotic nodules and lesions are considered reactive rather than pre-neoplastic [34].

The microscopic aspect revealed hepatocytes close to normal, with variations in size, bile stasis, iron and copper deposits, vacuolar degeneration and micro- or macrovesicular steatosis (Figure 1C).

Also, in regenerative nodules, although the reticulin network is preserved, the liver architecture is disordered with the presence of portal spaces and centrilobular veins inside nodules and also of fibrous tracts with well represented bile ductular reaction.

In our experience, the immunohistochemical analysis of sinusoidal capillarization with pan-endothelial markers as CD34 or CD31 indicated the higher microvascular density (MVD) in the periphery of regenerative nodule (Figure 1D). This immunohistochemical pattern of CD34 in regenerative nodules is indicated also by other literature studies and is considered useful for the differential diagnosis with high-grade dysplasia and HCC [34, 35].

Dysplastic nodules

The dysplastic lesions refer to the presence of atypical hepatocytes with abnormal histological characters and growth and without fulfilling the criteria for malignancy [34]. Dysplastic nodules are classified in low and high-grade dysplastic nodules [36].

The low-grade dysplastic nodules (LGDNs) have variable signal intensity on T1-weighted MR images, and are usually iso- or hypointense on T2-weighted MR images with enhancement similar to that of surrounding parenchyma after administration of gadolinium-based contrast material [31–33, 36].

The gross pathological appearance of LGDN is indistinguishable from those of regeneration, being similar to cirrhotic lesions, with low malignant potential. The presence of atypical hepatocytes groups, only visible microscopically, and that usually designates a lesion less than 1 mm is considered a dysplastic focus, and when the macroscopic or radiological lesion is visible and has over 1 mm, it is regarded as dysplastic nodule [34].

Microscopically, in the case of dysplastic nodules may be present large cell changes (visible nucleoli, hyperchromasia, increased sized nuclei, multiple nuclei, abundant cytoplasm) and small cell changes (reduced size, the higher nucleus to cytoplasm ratio, nuclear hyperchromasia) [37].

LGDNs are characterized by an intact reticulin framework, the hepatocytes have unremarkable cytology or low-grade atypia, portal tracts present in the nodules, and an increase in the number of unmatched arterioles.

In clinical practice, in the absence of clear criteria for histological high-grade dysplasia, any LGDN is considered a regenerative nodule [36]. There is no difference between the angiogenic immunohistochemical profile of regenerative nodules and LGDN [38].

The high-grade dysplastic nodules (HGDNs) are premalignant lesions and difficult to distinguish from well-differentiated HCC [31–33, 36].

In MR images, the signal intensity is variable on T1-weighted images, depends on their content, and usually iso- or hypointense on T2-weighted images. After contrast administration, they can show arterial enhancement similar to that seen in HCC, but usually they are hypovascular [31, 36, 39] (Figure 2A). Low- and high-grade dysplastic nodules and well-differentiated HCCs enhance after hepatobiliary and SPIO contrast agents, thus these contrast agents are not useful for the assessment of these lesions [22, 30]. Using DWI, approximately 80% of dysplastic nodules are iso- or hypointense, whereas a high percentage of HCCs are hyperintense [15] (Figure 2B).

Usually, the gross pathological appearance of HGDN is represented by nodules with imprecise edges and less than 1.5 cm in size [36].

The microscopic appearance of HGDN is represented by the presence of small cell changes, which along with the moderate increase of hepatocyte nuclear density, absence of atypical mitosis, reduced numbers of portal tracts, gradual sinusoidal capillarization, and chaotic arteries may be considered criteria for diagnosis and differentiation by HCC. In addition, the decrease of reticulin network, cellular cords thickness increase and pseudoglandular architecture can be seen in LGDN [34] (Figure 2C).
The immunohistochemical analysis of sinusoidal capillarization with pan-endothelial markers indicated the higher microvascular density (MVD) in the central compartment of HGDN (Figure 2D). The location of immunosignal and the higher CD34 MVD values compared with regenerative nodule is indicated by literature data [38] and in our experience, this aspect is useful for differential diagnosis. Literature data indicate the utility of immunohistochemical markers in differentiating HGDN by early hepatocellular carcinoma (eHCC) and well-differentiated hepatocarcinoma as heat shock protein 70, glutamine synthetase, glypican-3 and β-catenin [34].

Figure 1 – 3T MRI in a cirrhotic liver with regenerative nodules hyperintense in T1 (A), hypointense in T2 (B); Hematoxylin–Eosin (HE) staining, ×100 (C); CD34 immunostaining, ×100 (D).

Figure 2 – 3T MRI with gadobenate disodium showing dysplastic nodule in segment II and early inhomogeneous HCC in segment IV with high signal in T1 (A), low signal in T2 with fat suppression (B).
Hepatocellular carcinoma

The MR imaging features in hepatocellular carcinoma depending on grade, size, and biological properties and can be divided in two types, respectively small and large [40–42]. The size of small HCC is less than 2 cm, and in these cases, we talk about early small HCC, a relative new entity and MR features represented by a high signal in T1-weighted images, and hypo- or isointense on T2-weighted images. After contrast administration, the signal is hypo- or isointense in arterial phase and hypointense in portal phase. The recent developments in contrast agents specificity has increase the HCC diagnosis of all sizes [43, 44] (Figure 3A). Some well-differentiated small HCC may display a hepatobiliary enhancement because of residual hepatocyte, and this is one of the causes for false negative cases. After SPIO contrast agents administration these early lesions enhance similar to the surrounding parenchyma.

The prognostic for early HCC is very good, with no metastatic disease or vessel invasion, a rate of survival of 89% and recurrence after resection of only 8% [45]. The progressed small HCCs are similar with large HCCs as MRI features. The imaging features are round/oval lesions, with smooth contour, different signal intensity on T1-weighted images, and moderately hyperintense on T2-weighted images. After contrast administration, the enhancement is high in the arterial phase with washout in the delayed phases, becoming less intense than the surrounding parenchyma [41]. Metastases are present and the rate of survival is around 48% [36, 46].

The new guidelines recommend the diagnosis of small HCCs larger than 1 cm based on imaging findings (CT or MRI) when they have typical features, respectively hypersignal on arterial phase and washout in portal and delayed phase [39]. The lesions less than 1 cm in diameter, which are usually cirrhotic and stable, needs follow-up every three months with the same imaging method that was used to depict [39].

Recently, a classification of liver lesions was brought up-to-date (Liver Imaging Reporting and Data System/LI–RADS). The five classes are LR1 and LR5, definitely benign, respectively malign, LR2 and LR4, which can be used for lesions probably benign or malignant and LR3 for indeterminate nodules [47]. LI–RADS criteria provide a systematic approach to image analysis and reporting of insights in patients with cirrhosis or other risk factors for HCC. The implementation and continued adjustments of LI–RADS will enhance the care of patients with or at risk for HCC, lighten research and improve the field [47].

The rate of false negative results is relative high due to continuous morphologic and histopathological changes that produces inside high-grade dysplastic nodules or early HCC. Size is an important feature, the nodules smaller than 1 cm are probably benign and lesions larger than 2 cm are probably malignant, the difficulty is to classify the nodules between 1 and 2 cm. The high signal in T1- and T2-weighted images is suspicions for malignancy, as well as the arterial and capsular enhancement, internal mosaic structure and fatty transformation [48].

The MRI features of large HCC are characteristic, with hypointensity on T1-weighted images and heterogeneous hyperintensity on T2-weighted images. The heterogeneous appearance is due to presence of fat, hemorrhage or necrosis. The fat feature is signal loss in out-of-phase and fat saturated sequences. The hemorrhage has high signal on T1 images and low signal on T2-weighted images. Intralesional necrosis is characterized by hypointensity on T1-weighted images and hyperintensity on T2-weighted images. Another component is the HCC capsule that appears as a thin contour around the tumor, usually with low signal in T1- and T2-weighted images, and late enhancement after gadolinium administration [41, 42] (Figure 3B).

The HCC enhancement is in arterial phase with washout on portal and delayed phases. In rare cases, a large HCC may be hypovascular and in such cases is necessary biopsy for diagnosis. The enhancement of large HCC after hepatobiliary and SPIO contrast media is different, the poorly differentiated HCCs do not enhance, and thus contrast-enhanced sequences are useful. The uptake of well-differentiated HCCs is similar with the surrounding liver parenchyma, and may not be visible on contrast-enhanced sequences [49, 50].

The typical feature of HCC on DWI is a lesion with high signal intensity and $b$-values with restricted diffusion (dark) on ADC (apparent diffusion coefficient) image. The accuracy of differential diagnosis between dysplastic nodule and HCC increase by adding DWI to contrast enhanced sequences [51].
A large dysplastic or regenerative nodule may contain a focus of HCC a feature that characterize a nodule-in-nodule appearance. The signal intensity differs with no enhancement of dysplastic and regenerative lesion and enhancement of carcinomatous nodule. With the use of SPIO contrast media, the signal is high for carcinoma and low for regenerative and dysplastic nodule [52, 53].

The satellite lesions are common findings in hepatocellular carcinosomas, and they appear as multiple infra-centimeter nodules (Figure 3C). In addition, the invasion of the portal a hepatic veins with thrombosis is another feature of large HCCs, and the alterations of flow signal intensity is suggestive for the diagnosis. The malignant thrombus enhance in the early phases compared to a benign thrombus, which does not enhance. A diffuse infiltrating cancer with high risk of hematogenous spread may be diagnosed by the presence of tumor thrombus [54].

The differential diagnosis of hypervascular lesions includes transient arterial enhancing lesions, zones of confluent fibrosis or hemangiomas. An arteriopoortal shunting, pseudoaneurysm, the neovascularity, compression or thrombosis of portal veins are the main cause for transient arterial enhancing lesions. The usually appearance is that of an wedge shaped area of enhancement, isointense on T1- and T2-weighted images, peripheral in the case of arteriopoortal hunting or pseudoaneurysm, and segmental or lobar in portal thrombosis or compression. The neovascularity is revealed by an ill-defined area of enhancement around the malignancy [55]. The focal fibrosis appears as a wedge-shaped area, usually in the anterior and medial segments of liver, with low signal on T1-weighted images and high signal on T2-weighted images, and without enhancement in arterial phase but with delayed enhancement. It may mimic HCC in the case of arterial enhancement or when SPIO contrast is used. In the cirrhotic liver, the presence of hemangiomas is atypical, and the MR features are of a nodular mass with fibrosis, that does not wash out and is isointense in multiple phases [56].

Other rare conditions that may mimic HCC are focal nodular hyperplasia, hepatic adenoma, hypervascular metastases or intrahepatic cholangiocarcinoma, based on imaging and clinical history [57].

The gross appearance of the HCC depends on the size, presence of necrosis and hemorrhage, venous thrombus presence and intrahepatic metastasis [58] as well as liver cirrhosis and can be classified into diffuse, nodular and massive types [59–63]. Nodular type consists in the presence of one or more visible, well-defined nodules, while massive type is described as a tumor mass that replace volumes of hepatic parenchyma, infiltrative and poorly delimited [64]. In addition, the diffuse growth pattern appears as small nodules that can replace the entire liver parenchyma [64, 65].

The Japanese Study Group dedicated to HCC proposed a sub-classification of nodular type to introduce small tumors and the presence or absence of cirrhosis [66]. This classification of the nodular forms, which also are the most common forms, includes small nodular type, simple nodular type, simple nodular type with extranodular growth, and confluent multinodular type [60, 66]. Also, small nodular type, in which the size do not exceed 2 cm, it was distinguished as vague nodular and nodular distinct types [36].

These classifications have been found to have a prognostic impact, so that the nodular type appears to be less aggressive compared to the diffuse and massive types [64]. At the same time, the existence of a capsule in solitary nodules gives a better prognosis compared with those with parenchymal or blood vessel invasion, or microsatellite metastatic tumor formation, characteristic aspects for massive, diffuse and extranodular growth gross types [67–69].

There are described particular macroscopic aspects of HCC, as pedunculated form which appears as an extrahepatic tumoral mass linked (subtype I) or not (subtype II) through a pedicle by the liver surface [62, 63, 70]. Other HCC macroscopic particular aspects are present in scirrhous HCC (stellate fibrosis), fibrolamellar HCC (fibrous tracts forming central scar), peliosis hepatis-like HCC (hemorrhagic nodular areas) [60, 71]. In these cases, imaging investigations may be difficult to interpret due to fibrosis and hemorrhagic areas. Such changes may be present after chemotherapy, irradiation and chemoembolization, or in case of intrahepatic cholangiocarcinoma or metastatic carcinoma [63].

On the contrary, imaging investigations are absolutely necessary for particular forms, as is the case with HCC with intraductal biliary growth pattern, which is difficult to assess even in the case macroscopic analysis on hepatectomy specimens [71, 72].

The microscopic aspects of small eHCC indicate well-differentiated HCC with normal cells replaced by neoplastic cells. The early HCC (macroscopic corresponding to small vague nodule) and characterized by stromal invasion, respectively the presence of hepatocytes groups in the portal tracts, fibrous septa or liver parenchyma and in which the ductular reaction is absent [73]. In addition, the portal tracts are reduced in number; there are insufficient arteries that produce low blood supply and a cellular concentration, which concur to hypoxia [46]. The relative hypoxia induced can be the explanation of steatotic transformation that occurs in a high percentage of people with early HCC [45].

On the contrary, progressed HCC, it is typically a malignant moderately differentiated lesion, being capable of vascular invasion and metastasis [36, 64].

In the case of HCC, the histopathological evaluation criteria are primarily the architectural and cytological aspects that are essential for HCC tumor differentiation degree. The reticulum network is destroyed. The microscopic appearance of HCC malignant cells keeps the features of hepatocytes (in most cases the appearance of the cords of hepatocytes is preserved) [63]. However, these basic features can only be found in certain areas of the tumor, architecture varying significantly between different tumors, and even within the same HCC (Figure 3D).

Cytoarchitectonic variants

Classic pathology describes several forms of cyto-architectonics for conventional HCC. These include the trabecular pattern (sinusoidal), the pseudoglandular (acinar or adenoid) pattern, the solid (compact) pattern, and the scirrhous pattern [63].

Trabecular type is composed of well-differentiated trabeculae consisting of five to eight cell layers, being covered by flattened endothelial cells and the features of small cell change are present (Figure 3E). Occasionally,
trabeculae composed of eight cell layers (macrotrabecular type) can be seen. The aspect in this case is most often cavernous vascular spaces filled with blood. Usual, the reticulin framework and Kupffer cells are absent.

The pseudoglandular (acinar, adenoid) type consists of gland-like spaces or acini lined by tumoral hepatocytes, formed by the dilatation of the bile ducts, which may contain bile or dense eosinophilic material representing inflammatory debris (Figure 3F). Sometimes sinusoidal and the pseudoglandular types can coexist and gland-like spaces may occur through the trabecular structures cystic degeneration [63]. These first two types of HCC are the most common models of well-differentiated HCCs.

The compact type is usually observed in the case of a poorly differentiated HCC; trabeculae are still present, but are poorly represented and often disorganized. The tumor appears to be composed of layers of small cells of the vascular space adjacent to the compressed cellular material (Figure 3G).

Scirrhouus type is an uncommon HCC type. It presents fibrous septa marked by compact groups of malignant cells. The fibrosis can be focal or diffuse and this HCC type can be associated with sinusoidal, pseudoglandular or solid types [63]. Although this variant is usually seen post-irradiation or chemotherapy, it can occur in the absence of these factors. It is also described the HCC sclerosing variant, in which the fibrosis is marked [63].

Other special HCC histological types, some recognized by the WHO (World Health Organization) as cytological forms, includes fibrolamellar, lymphoepithelioma-like, sarcomatoid and undifferentiated types [34].

The fibrolamellar type presents large polygonal cells with abundant eosinophilic granular cytoplasm, nuclei with macronucleoli and thin hyalinized collagen fibrosis deposits; tumor cells or small cords are arranged in trabeculae. Malignant cells show abundant mitochondria, eosinophilia giving them specific and clear cytoplasm with dense inclusions.

Lymphoepithelioma-like HCC is a rare subtype with syncytial growth pattern, pleomorphism, lymphocyte infiltration in the tumor and implication of Epstein–Barr virus in etiology [34]. Sarcomatoid type of HCC presents an epithelial component and a component of malignant spindle cells that cannot be distinguished from a sarcoma, with transition areas between the two components, immunohistochemistry for cytokeratin and vimentin being usually positive [34].

Some of the issues described may be present after chemotherapy or transcatheterin arterial embolization, which of can cause phenotypic changes.

**Cytological variants**

Various cytological aspects of HCC are described. Cells showed predominantly classic aspects with similar morphology of hepatocytes, polygonal, vesicular, round nuclei and prominent nucleoli. The ratio between the nucleus and the cytoplasm, as well as the degree of pleomorphism and hyperchromatism varies with the degree of differentiation of the tumor.

Along with small cell changes, may be present large cell changes and giant or pleomorphic cells. The granular appearance of cytoplasm due to the number of mitochondria and decreased basophilia compared with normal hepatocytes are characteristic issues (Figure 3H).

Pleomorphic variants contain cells, which are usually larger and contain multiple nuclei of bizarre shapes, sometimes with osteoclast-like appearance. These cells rarely form compact masses, are discohesive and characteristic for poorly differentiated HCC (Figure 3I).

Clear cell variants of HCC show abundant, clear cytoplasm, due to abundant deposits of glycogen, fat or water (Figure 3J). The nuclei are usually centrally located. Clear cells predominate in the tumor, and usually grow on trabecular cords. Sometimes this appearance can be mistaken for with metastatic clear cell carcinoma [34, 63]. Although this type of cell was associated with a better prognosis, there is no convincing evidence for this theory [68, 69]. Focally aspects of small cell, giant cell, sarcomatoid, oncocytoid or rhabdoid appearance may be present in the case of conventional HCC.

**Cytoplasmic inclusions**

HCC tumor cells present several cytoplasmic inclusions. Triglyceride cytoplasmic inclusions are visible especially for tumors less than 2 cm in size [63].

Mallory bodies are PAS-negative masses of intermediate filaments, visible in electron microscopy. Pale bodies are frequent in fibrolamellar hepatocarcinomas and contain fibrinogen, representing fibrillar structures within dilated endoplasmic reticulum [74]. PAS-positive granular hyaline bodies of different sizes can be seen in about 15% of HCCs, with both intra- and extracellular locations. Following immunohistochemical analysis, it was demonstrated that they contain liver products such as albumin, alpha-fetoprotein, alpha-1 antitrypsin, bile or ferritin [75]. Mallory bodies, by content of abnormal keratins, ubiquitin, and heat shock proteins differs of intra- and extracellular hyaline bodies, although their coexistence is demonstrated in HCC [76]. Although HBsAg within hepatocytes confers a characteristic “ground-glass” aspect, this is not a characteristic for HCC being considered tumoral entrapped HBsAg hepatocytes [34, 63]. Sometimes was described in the cytoplasm of tumor cells the presence of a melanin-like, dark brown pigment, similar to Dubin–Johnson syndrome [34].

HCC grading takes into account the cytological and architectural aspects. Thus, in well-differentiated HCC, the pattern is usually trabecular or acinar, sizes less than 3 cm and steatosis is present. By comparison, in the case of moderately differentiated HCC, the trabecular aspect is weak, with trabeculae thicker and increased cytological atypia. In poorly differentiated and undifferentiated tumors, the solid infiltrative growth pattern with sarcomatoid differentiation, pleomorphism and marked atypia are characteristic.

HCC heterogeneity in regarding the degree of differentiation and the impossibility of objective evaluation on biopsy fragments, makes this histological parameter do not always provide essential information about prognosis [64]. The main morphological prognostic parameters for HCC remain tumor stage (size and number of tumors, vascular and extrahepatic invasion) [64].

**Immunohistochemical markers** in HCC are useful for the differential diagnosis both with dysplastic nodules in case of well HCC and other tumors (hepatocellular neoplasms, adenocarcinomas, and metastasis) in case of poorly differentiated HCC.

Heat shock protein 70, glutamine synthetase, glypican-3
and β-catenin expression shows differences in regenerative/dysplastic lesions compared with early and well-differentiated HCC, and can be used for differential diagnosis [34, 63]. From our experience, we observed that CD34 MVD can differentiate between regenerative nodules/LGDN and HGDN/HCC through both immunostaining pattern (peripheral vs. central) and the values significantly higher in high-grade dysplasia lesions and HCC (Figure 3K). Furthermore, new methods were used to characterize the liver tumor vasculature. Thereby, some methods of fractal analysis of primary and metastatic liver tumor vasculature, as well as in non-tumor lesions, can be used in order to differentiate lesions [77].

HepPar 1 has a high sensitivity for HCC and is the most used marker, although it can be negative in poorly differentiated HCC and scirrhou forms, and positive in some digestive or lung adenocarcinomas [34, 78, 79].

Another useful marker is polyclonal CEA, indicating a canalicul pattern in case of HCC, cytoplasmic in other carcinomas and usually negative for HGDN [34] (Figure 3L).

Figure 3 – (A) 3T MRI Ax T2, using Gd–EOB–DTPA and revealing a small hypovascular HCC in segment VII; (B) 3T volume reconstruction large HCC with inhomogeneous internal appearance with necrosis and thin capsule; (C) A satellite nodule hyperintense in ax T2 with fat suppression; (D) HCC, trabecular and pseudoglandular mixed pattern, HE staining, ×100; (E) HCC, trabecular type, HE staining, ×100; (F) HCC, pseudoglandular type, HE staining, ×100; (G) HCC, compact type, HE staining, ×100; (H) Hepatic residual parenchyma and carcinoma with trabecular pattern, HE staining, ×100; (I) HCC, pleomorphic variant, HE staining, ×100; (J) HCC, clear cell variant, HE staining, ×100; (K) HCC, CD34 immunostaining, ×100; (L) HCC, CEA-specific canalicul immunostaining pattern, ×100.
Literature data also indicate α-fetoprotein as specific but less sensitive for HCC, MOC1 negativity and CAM 5.2, CK7, CK19, CK20 positivity useful for the differential diagnosis of HCC [34]. Arginase-1, HepPar 1 and glypican-3 seem to be the best markers for distinguishing HCC from metastatic tumors [80]. Panels of immunohistochemical markers along with the morphological and imaging features of regenerative nodules, dysplastic nodules and HCC are required for assessing prognosis and therapeutic management of the lesions (Table 1).

### Table 1 – Comparative diagnosis features for the hepatic nodular lesions

<table>
<thead>
<tr>
<th>Parameter/Lesion</th>
<th>Regenerative nodules</th>
<th>LGDN</th>
<th>HGDN</th>
<th>HCC</th>
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<tbody>
<tr>
<td>MRI T1 weighted</td>
<td>variable (high for protein/lipids content)</td>
<td>variable</td>
<td>variable</td>
<td>• high (small early HCC) / variable (small progressed HCC) / hypointense (large HCC)</td>
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<tr>
<td>MRI T2 weighted</td>
<td>iso-/hypointense</td>
<td>iso-/hypointense</td>
<td>iso-/hypointense</td>
<td>• iso-/hypointense (small early HCC) / heterogeneous hyperintense (small progressed HCC and large HCC); hyperintense</td>
</tr>
<tr>
<td>DWI</td>
<td>iso-/hypointense</td>
<td></td>
<td></td>
<td>• variable in size, well defined or imprecise edges; diffuse, nodular and massive types; necrosis, hemorrhage.</td>
</tr>
<tr>
<td>Macroscopy</td>
<td>• 1–3 cm, well defined; green or yellow-brown.</td>
<td>• 1–3 cm, well defined; green or yellow-brown.</td>
<td>&lt;1.5 cm, imprecise edges</td>
<td>• reticulin network preserved; • hepatocytes close to normal; • liver architecture disordered; • ductular reaction.</td>
</tr>
<tr>
<td>Microscopy</td>
<td>• reticulin network preserved; • hepatocytes close to normal; • liver architecture disordered; • ductular reaction.</td>
<td>• reticulin network preserved; • hepatocytes close to normal; • liver architecture disordered; • ductular reaction.</td>
<td>• decrease of reticulin network; • small and large cell changes; • liver architecture disordered; • ductular reaction.</td>
<td>• reticulin network destroyed; • stromal invasion; • malignant cytology; • no ductular reaction.</td>
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<tr>
<td>Immunohistochemistry</td>
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<tr>
<td>Sinusoidal capillarization</td>
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<tr>
<td>Malignant vs. non-malignant</td>
<td>Heat shock protein 70, glutamine synthetase, glypican-3, β-catenin</td>
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<tr>
<td>Malignant vs. metastasis</td>
<td>HepPar 1, polyclonal CEA, arginase-1, glypican-3, CAM 5.2, CK7, CK19, CK20</td>
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</tbody>
</table>

LGDN: Low-grade dysplastic nodule; HGDN: High-grade dysplastic nodule; HCC: Hepatocellular carcinoma; MRI: Magnetic resonance imaging; DWI: Diffusion-weighted imaging; MVD: Microvascular density; HepPar 1: hepatocyte paraffin 1; CEA: Carcinoembryonic antigen; CAM 5.2: Anti-cytokeratin (CAM 5.2) mouse monoclonal primary antibody; CK: Cytokeratin.

Highlighting and analyzing these differences can be the basis of further classification of nodular lesions in the liver. For instance, a prognostic factor for the future, may be represented by determining the molecular classification of HCC, which may be the gateway to a targeted and effective treatment; today were identified two target groups based on etiology, genes and proteins involved in HCC, one of these with predominantly proliferative mechanisms, and another with a central role of β-catenin [81, 82].

The characterization of liver lesions in the progression of tumor, it is important for the understanding of liver carcinogenesis both in the imaging and histopathological analysis [83]. Imagistic-pathological studies are important to standardize criteria in non-invasive diagnostic imaging, especially for small HCC or in case of particular aspects [83–85].

### Conclusions

Hypervascular nodules in cirrhotic liver are a group of lesions that may be classified histologically as regenerative (benign) or as dysplastic or neoplastic (prenmalignant or malignant). For an accurate diagnosis, a correlation between the clinical, pathological and imaging features is necessary. Moreover, a comprehensive familiarity with specific features at unenhanced and contrast-enhanced MR imaging may help distinguish benign lesions from premalignant and malignant ones, for a better management and follow-up of HCC.

**Conflict of interests**

The authors declare that they have no conflict of interests.

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