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# The Histomorphological and Immunohistochemical Diagnosis of Hepatocellular Carcinoma

Daniela Fanni, Clara Gerosa and Gavino Faa
*University of Cagliari*
*Italy*

## 1. Introduction

Hepatocellular carcinoma is one of the most common malignancies worldwide, and its incidence is growing in association with viral and nonviral chronic liver diseases (Sherman M, 2010). Hepatocellular carcinoma is a lethal cancer, and improved survival relies on the detection of early tumors smaller then 2 cm, which are less likely to have dissemination (Di Tommaso, 2011). The diagnosis of hepatocellular carcinoma is based on a multidisciplinary approach, including imaging modalities and serum markers, such as alphafetoprotein (Zhou L 2006). However, such diagnosis still rests on the incontrovertible histological evidence obtained by CT scan or echo-guided needle biopsy. Radiology is the main technique used to detect hepatocellular carcinoma in the setting of cirrhosis; the typical imaging shows hepatocellular carcinoma bigger then 2 cm in more than 90% of cases. When the radiological features of hepatic liver nodules in cirrhosis are not typical, the American Association for the Study of Liver Diseases (AASLD) guidelines recommend the use of liver biopsy. The potential risks of bleeding and seeding due to liver biopsy can be minimized with very thin, 20- to 21-gauge needles, so this invasive technique is recommended now and will be recommended in the future for approximately 50% of dubious hepatic nodules between 1 and 2 cm in size. Indeed, the cumulative experience of several internationally recognized hepatology centers (Bolondi L, 2005)(Forner A, 2008)(Sangiovanni A, 2010)(Leoni S, 2010) has shown that 30% of 1 to 2 cm nodules are not malignant. Recently, Leoni et al. have reported that the application of international guidelines, even with three imaging techniques, leads to a false-negative rate of 20% for nodules ultimately shown to be hepatocellular carcinoma (Leoni S, 2010), whereas Sangiovanni et al. (Sangiovanni A, 2010) have documented in a single-technique scenario that 55% of patients will need to undergo biopsy for a final diagnosis. Thus, biopsy has a very crucial role when radiology fails and pathologists are asked to document malignancies in lesions that are also the most difficult to ascertain (Sherman M, 2010). Regarding the application of needle biopsy for small liver nodules, the American Association for the Study of Liver Diseases recommends that biopsy should be performed for nodules less than 2 cm if their radiologic findings are not characteristic of hepatocellular arcinoma, whereas biopsy is not needed for lesions showing characteristic radiologic findings (Bruix J, 2005). This recommendation has been supported by prospective validation (Forner A, 2008) (Bruix J, 2011). Core liver biopsy is definitely

superior to fine needle aspiration, because the specimen obtained is suitable for the assessment of both architectural and cytologic features. Furthermore, the tissue block obtained provides materials for marker studies. Fine needle aspiration is usually adequate for the evaluation of large lesions that are likely to be moderately to poorly differentiated, where diagnostic criteria are easier to evaluate (ICGHN, 2009). An International Working Party of the World Congresses of Gastroenterology proposed a consensus nomenclature and diagnostic criteria for hepatocellular nodular lesions (International Working Party, 1995). The International Working Party classified nodular lesions found in chronic liver disease into large regenerative nodule, low-grade dysplastic nodule, high-grade dysplastic nodule, and hepatocellular carcinoma; this nomenclature has been widely adopted. In addition, the International Working Party introduced the concept of dysplastic focus as a cluster of hepatocytes with features of early neoplasia (in particular small cell change or iron-free foci in a siderotic background) measuring less than 0.1 cm, and defined small hepatocellular carcinoma as a tumor measuring less than 2 cm (International Working Party, 1995). More recent studies support the division of small hepatocellular carcinoma into two clinico-pathological groups that have been termed early hepatocellular carcinoma and progressed hepatocellular carcinoma. Early hepatocellular carcinoma has a longer time to recurrence and a higher 5-year survival rate compared with progressed hepatocellular carcinoma (ICGHN, 2009).

## 2. Morphological features

The histological appearance of hepatocellular carcinoma has been described in detail over the years and criteria for diagnosis and nomenclature have been clarified (Lopez JB 2005). Distinct morphological features have been reported and, in the majority of cases, hepatocellular can be identified by routine haematoxylin-eosin stained sections, that show a malignant lesion with evidence of hepatocellular differentiation (ICGHN, 2009). The hepatocellular carcinoma's cells are variably similar to the normal liver cells, depending on the degree of differentiation (Ishak KG, 2001). The nuclei are usually hyperchromic and irregular with prominent nucleoli and a high nuclear/cytoplasmic ratio. The tumor cells usually have distinct cell membranes and a moderate amount of eosinophilic, finely granular cytoplasm and may contain a variety of cellular products, mimicking normal and pathologic liver cell function (Goodman ZD, 2007). The cytoplasm could give a clear cell appearance due to the presence of cytoplasmic fat and/or glycogen. Cytoplasmic Mallory bodies and hyaline globules, representing alpha-1-antitrypsin storage, lightly eosinophilic ground-glass-like cytoplasmic inclusions, due to fibrinogen and other plasma proteins can be found. Bile pigment may be present in tumor cells or in dilated canaliculi, that can be easily observed even in hematoxylin eosin stained sections (Goodman ZD, 2007). The cells of hepatocellular carcinoma generally try to grow in ways that mimic the cell plates of normal liver, producing well-recognized growth trabecular pattern (Ishak KG, 2001). The histological picture could remember the early architecture of embryonic and foetal liver (fig. 1), suggesting the hypothesis that carcinogenesis follows liver developing ontogenesis. The architectural feature show different growth patterns. Most often the tumor grows in a **trabecular pattern** with thickened cords of cells separated by vascular sinusoids, mimicking the cell plates and sinusoids of normal liver; a rapid growth of the tumor cells causes the plates to become thickened and contorted, producing trabeculae that are surrounded by endothelial cells. The trabeculae may contain very dilated canaliculi, producing a

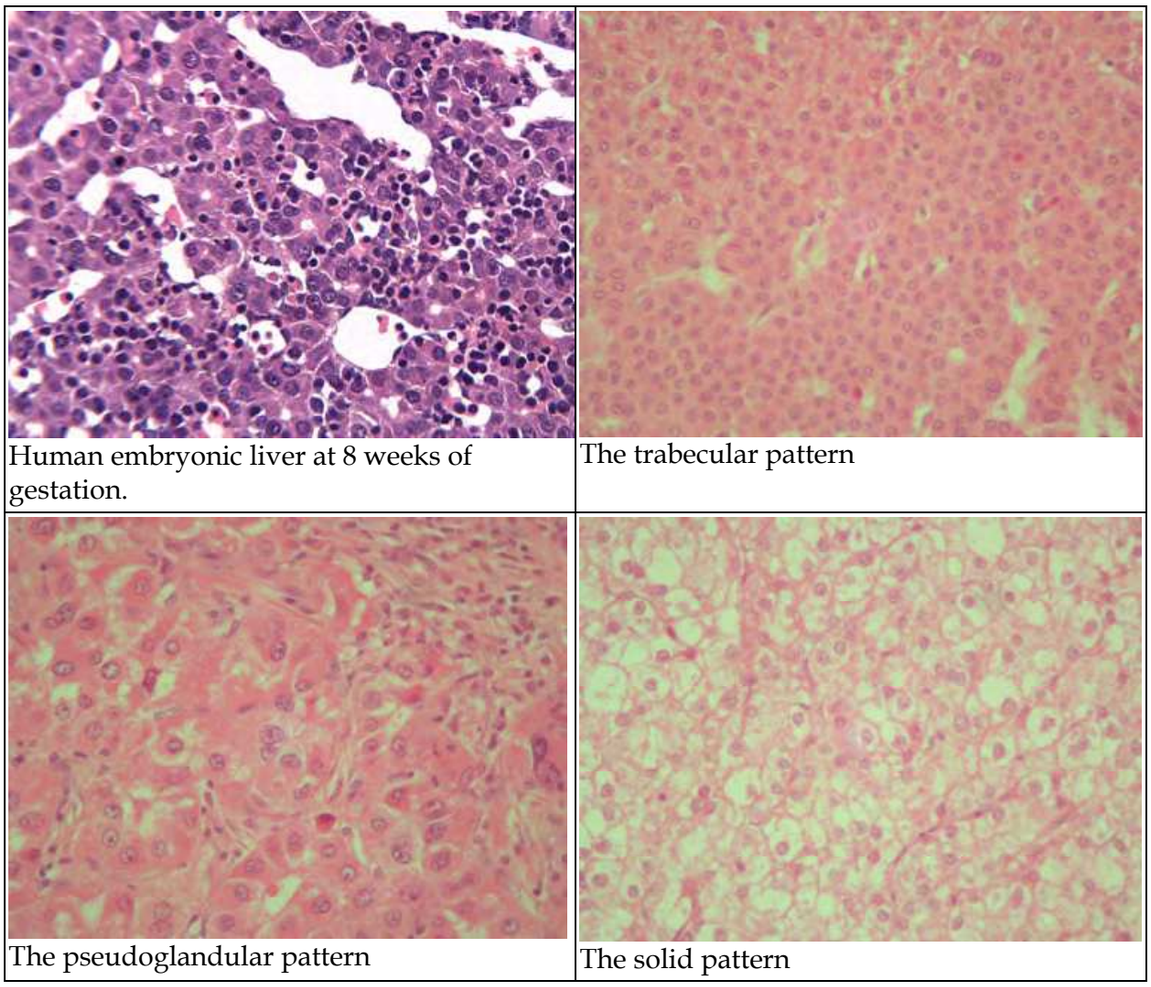



Fig. 1. The architecture of a human embryonic liver at 8 weeks of gestation compared with the growth patterns of hepatocellular carcinoma.

**pseudoglandular pattern**, in other fields, trabeculae may grow together, compressing the sinusoids and forming sheets of tumor cells, producing a **solid or compact pattern** (Goodman ZD, 2007). Early hepatocellular carcinoma has a vaguely nodular appearance and is well differentiated. Progressed hepatocellular carcinoma has a distinctly nodular pattern and is mostly moderately differentiated, often with evidence of microvascular invasion (ICGHN, 2009). Small lesions with malignant potential show only subtle differences from the surrounding parenchyma, making them difficult to assess reproducibly. The International Working Party criteria of 1995 have led to remarkable progress in global standardization of nomenclature of liver nodules (International Working Party, 1995). However, although these criteria have been widely adopted, their application is challenging in equivocal lesions. Perhaps the most significant problem is that most histologic criteria are arrayed on a gradual spectrum and cannot be easily summarized as "present or absent". Additionally, the number of criteria suggested in the literature are too numerous to achieve interobserver consensus, and the diagnostic weight carried by each of these criteria is uncertain. Frequently, criteria used for malignancy in other tissues, such as mitotic activity

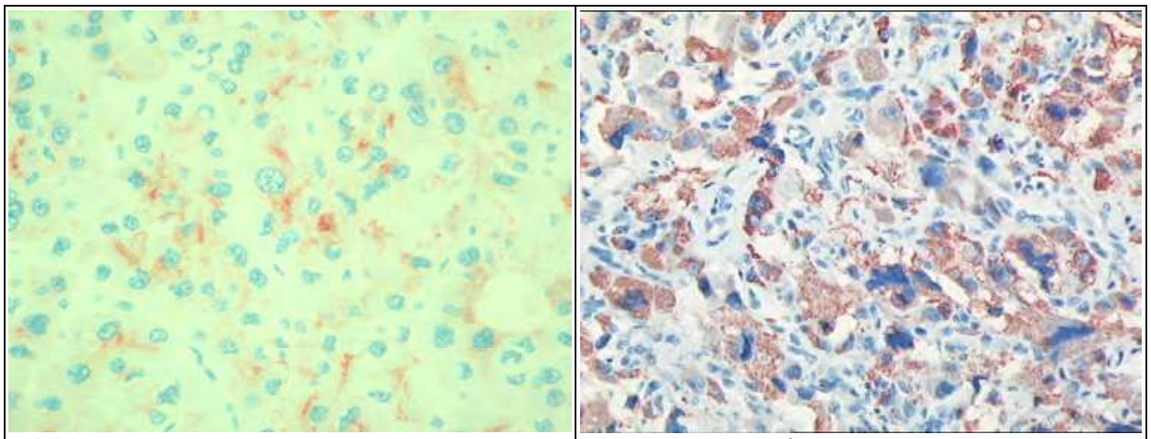
and cellular atypia, are not represented to a significant degree in well differentiated hepatocellular carcinoma. In addition, the liver lacks a layered structure as seen in the gastrointestinal tract, it is difficult to determine the presence of destructive growth in early hepatocellular carcinomas. Despite these difficulties, current histologic criteria for these nodules clearly yield reliable diagnoses at both ends of the spectrum; most pathologists will correctly identify nodules up to low grade dysplastic nodule as benign, whereas even small well differentiated hepatocellular carcioma with distinct nodular pattern or small moderately differentiated hepatocellular carcinomas will be correctly identified as malignant. The remaining gray zone includes high grade dysplastic nodule and early hepatocellular carcinoma. In evaluation of these lesions, the presence of stromal invasion is a useful criterion of malignancy. Accordingly, pathologists can decide whether the equivocal tumor is hepatocellular carcinoma or high grade dysplastic nodule by recognizing the presence or absence of tumor cell invasion into the intratumoral portal tracts. When obvious stromal invasion is not found in an equivocal tumor, the lesion may be diagnosed as either high grade dysplastic nodules or early hepatocellular carcinomas without detectable invasion (ICGHN, 2009). The diagnosis of stromal invasion is subjective and may require the assistance of histochemical (Victoria Blue or reticulin stains) and immunohistochemical stains (keratin 7 or 19) for differentiation from pseudoinvasion (Park YN, 1998). Biopsy diagnosis of equivocal nodules remains a challenge, because minute biopsy specimens may not contain intratumoral portal tracts, thus precluding the detection of stromal invasion. Similarly, the detection of unpaired arteries, mitoses, and various immunohistochemical markers are prone to sampling error (Senes G, 2007). Connective tissue stroma is typically sparse, and reticulin fibers are absent or reduced, being found only at the periphery of trabeculae. In contrast to most other malignant epithelial neoplasms, hepatocellular carcinoma is soft and the lack of a desmoplastic stroma is a helpful diagnostic clue (Goodman ZD, 2007). A subset of tumors, that has been named **fibrolamellar epatocellular carcinoma**, differs from other types of hepatocellular carcinoma in clinical features and prognosis (Craig JR, 1980)(Soreide O, 1986). Fibrolamellar carcinoma is a unique type of liver carcinoma that arises in non-cirrhotic livers of young individuals (Torbenson M, 2007). Its etiology is unknown. Histologically, the tumor is made up of large polygonal cells characterized by abundant granular eosinophilic cytoplasm, large vesiculated nuclei, prominent nucleoli and abundant stroma composed of lamellae of collagen. Lamellar bands of fibrosis are present within the tumor and can be seen in both primary and metastatic tumors (Ross HM, 2011). Rarely, hepatocellular carcinoma will produce abundant stroma, producing the scirrhous pattern of hepatocellular carcinoma (Goodman ZD, 2007).

## 3. Immunohistochemistry

Immunostains can be used to study hepatocellular carcinoma and to distinguish hepatocellular carcinoma from other focal lesion or other malignancies, especially intrahepatic cholangiocarcinoma and metastatic adenocarcinoma (Ishak KG, 2001)(Varma V, 2004). As the cells of hepatocellular cacinoma attempt to mimic normal liver cells, they may produce any of the cellular products that can be found in hepatocytes both in health and in disease, and, if present, these are readily demonstrated by immunostaining. Unfortunately, they are not specific and many of these can also be found in tumors other than

hepatocellular carcinoma (Goodman ZD, 2007). There are no stains that can absolutely distinguish well-differentiated hepatocellular carcinoma from hepatic benign lesions, such as hepatocellular adenoma or dysplastic nodules, and similarly, no single stain can always distinguish poorly differentiated hepatocellular carcinoma from poorly differentiated cholangiocarcinoma or metastatic adenocarcinoma. However, selected immunostains, taken in the context of other morphologic features, can be very helpful in establishing the diagnosis of hepatocellular carcinoma in difficult cases.

The **polyclonal antiserum to carcinoembryonic antigen (pCEA)** is useful in demonstrating bile canaliculi (fig. 2), both in normal liver and in hepatocellular carcinoma, due to the presence of a CEA-like cross-reactive substance called biliary glycoprotein I (Saad RS, 2004)(Goodman ZD, 2007) (Onofre AS, 2007) (Lau SK, 2002).



pCEA reactivity | Hep-Par 1 granular pattern reactivity.

Fig. 2. Immunohistochemistry for pCEA and Hep-Par 1.

**HepPar-1 (Hepatocyte Paraffin 1)** is a monoclonal antibody that reacts with an epitope of liver mitochondria, with a typical granular pattern (fig. 2) in most liver specimens (Lugli A, 2004)(Minervini MI, 1997) (Leong AS , 1998) (Zimmerman , 2001). It also sometimes reacts with other normal or pathological structure, such as renal tubules and intestinal epithelium as well as with intestinal metaplasia in the stomach and esophagus (Goodman ZD, 2007). It produces positive staining in the vast majority of cases of hepatocellularr carcinoma (Saad RS, 2004)(Siddiqui MT, 2002) and only a small percentage of other tumors, including some cholangiocarcinomas and metastatic adenocarcinomas from the stomach and other sites (Terracciano LM, 2003), but when used in the context of morphology, clinical setting and other stains, HepPar-1 is very useful in distinguishing hepatocelluar carcinoma from other malignancies (Wang L, 2006). The scientific literature reported cases of hepatocellular carcinoma negative for HepPar-1 (Sugiki T, 2004), probably due to the uneven distribution of HepPar-1 in hepatocellular carcinoma, as reported by our group (Senes G, 2007). What's more the degree of positive staining could varies from case to case (Goodman ZD, 2007): some tumors have a very patchy distribution of positive cells, which can be easily missed in a small biopsy.

**Glypican 3** (GPC3), a cell-surface heparan sulfate proteoglycans that is secreted into the plasma, is a member of the glypican family of heparinsulfate proteoglycan linked to the cell

surface through a glycosylphosphatidylinositol anchor (Filmus J, 2001). Glypican 3 has been suggested to play a negative role in cell proliferation and an apoptosis-inducing role in specific tissues (Cano-Gauci DF, 1999). The gene is frequently methylated in various tumors and cell lines, suggesting a tumor-suppressive role in tumorigenesis (Di Tommaso, 2007). It is also overexpressed in hepatocellular carcinoma (Zhu ZW, 2001). Glypican-3 has recently become established as a serum and tissue marker for hepatocellular carcinoma, both at messenger and protein level (Capurro, 2003)(Libbrecht L 2006) (ICGHN, 2009). At the beginning, by using a home-made antibody, glypican 3 weak and focal staining was observed in hepatocellular carcinoma precursor lesions and a diffuse staining in the vast majority of hepatocellular carcinomas (Yamauchi N, 2005). Glypican-3 immunoreactivity has a reported sensitivity of 77% and specificity of 96% in the diagnosis of small hepatocellular carcinoma; therefore, glypican-3 positivity is a strong argument for malignancy but the absence of immunoreactivity does not help in the differential diagnosis (Libbrecht L 2006)(ICGHN, 2009). The staining pattern is usually cytoplasmic but may be membranous or canalicular. The monoclonal antibody from Biomosaics (IG12 clone) at a dilution of 1:50 to 1:100 as amplified with the new short polymer systems (Advance [Dako], Novolink [Novocastra], and Super-picture + [Zymed]) yields reliable results. Since glypican-3 staining may be only focal, additional markers or a panel of markers may be necessary. Glypican-3 staining must be interpreted in context, because it may also be seen in regenerating hepatocytes in a setting of hepatitis and in melanocytic lesions (ICGHN, 2009). A recent work from our group (Sollai M, 2011) sowed immunoreactivity for glypican-3 in the fetal human liver, caracterized by a strong immunostaning during the first month of gestation and by a progressive decrease, ending with a very weak expression at birth. This work may add new data to the theory of strict similarities between cell programs utilized during development, silenced at birth, and re-activated by tumor cells during liver cancerogenesis.

**HSP70** (heat shock protein 70) belongs to a class of genes (heat shock proteins) implicated in tumorigenesis, in the regulation of cell-cycle progression and apoptosis (Garrido C, 2001)(Helmbrecht K, 2000)(ICGHN, 2009). Most hepatocellular carcinomas are associated with chronic inflammation and fibrosis, acting as stressful conditions that lead to heat shock protein synthesis. HSP70 is, in particular, a potent antiapoptotic survival factor (ICGHN, 2009); it was reported as the most abundantly up-regulated gene among a set of 12,600 genes in early hepatocellular carcinoma (Chuma et al, 2003). Furthermore, it was significantly overexpressed in progressed hepatocellular carcinoma as compared with early hepatocellular carcinoma, and in the latter as compared with precancerous lesions. HSP70 immunoreactivity was recently reported in the majority of hepatocellular carcinomas, including early and well-differentiated forms, but not in non-malignant nodules (Di Tommaso L, 2007), thus suggesting its use as a marker of malignancy. HSP70 immunoreactivity (SC-24 clone, dilution 1:250 to 1:500 amplified with short polymer systems; Santa Cruz Biotechnology, Santa Cruz, CA) is nucleocytoplasmic and mostly focal with 70% sensitivity for hepatocellular carcinoma detection in surgically resected specimens (Haussinger D, 1985).

**GS** (glutamine synteatse) catalyzes the synthesis of glutamine from glutamate and ammonia in the mammalian liver (Haussinger D, 1985) where it has been shown to be restricted to hepatocytes surrounding the terminal hepatic venules in the murine and human liver

(Moorman AF, 1988, 1989). It is known that glutamine, the end product of glutamine synteatse activity, is the major energy source of tumor cells (Reitzer LJ, 1979). Based on findings on experimental hepatocarcinogenesis, GS positive tumor cells are believed to be derived from GS positive hepatocytes that have been affected by carcinogens. Thus, GS was suggested as a specific marker for tracing cell lineage relationships during hepatocarcinogenesis (Gebhardt R, 1999). Most importantly, GS is a target gene of β-catenin so that its overexpression is associated with mutations of β -catenin or with activation of this pathway (Christa et al, 1994)(ICGHN, 2009). Then up-regulation of GS mRNA, protein, and activity has been demonstrated in human hepatocellular carcinoma (Christa et al, 1994). Interestingly, a stepwise increase in GS immunoreactivity from precancerous lesions to early and to advanced hepatocellular carcinoma was also reported, proposing for GS a role in the promotion of the metastatic potential of hepatocellular carcinogenesis (Osada et al, 1999). The monoclonal antibody from Chemicon International (clone MB302) at a dilution of 1:500 to 1:1000 and amplified with a new short polymer system yields reliable results. In order to increase its specificity as a marker of malignancy, GS immunostaining should be diffuse and strong, a pattern that can be seen in 50% of hepatocellular carcinomas, including early forms (Haussinger D, 1985).

**CHC (clathrin heavy chain)** is an endothelial marker overexpressed in hepatocellular carcinoma (Seimiya M, 2008). CHC was chosen because it is an endothelial marker, it works well as an internal standard for non parenchymal liver cells and, as already suggested in a surgical series, is overexpressed in the cytoplasm of malignant hepatocytes (Seimiya M, 2008). In contrast, most non malignant hepatocytes were reported to be negative or to have weak to moderate staining intensity (Seimiya M, 2008).

**Alpha-fetoprotein** is frequently elevated in the serum of patients with hepatocellular carcinoma, even when the tumor is negative at immunostaining (Zhou L, 2006)(Wang L 2006). In most published series, it has been found in less than an half of hepatocellular carcinomas: for that reason, it is less useful for diagnostic purposes (Goodman ZD, 2007) (Chu PG, 2002) (Onofre AS , 2007) (Lau SK , 2002).

**Cytokeratins** are sometimes suggested as a means of tumor classification, especially **cytokeratin 7** (Maeda , 1996)(Chu , 2000)(Durnez A et al 2006) and **cytokeratin 20** (Chu P, 2000) (Faa G , 1998), but in rare cases the tumor cells display aberrant keratin expression, suggesting that the staining pattern should be interpreted with caution (Fanni D, 2009). Cytokeratin 7 can be present in some hepatocelluar carcinomas, as well as cytokeratin 20, the latter in a minority of case. For that reason, cytokeratin 7 and 20 profiles are not very useful in the diagnosis of hepatocellular carcinoma (Goodman ZD, 2007). **Cytokeratin 8-18** (Porcell , 2000) (Van Eyken P, 1988), **cytokeratin 19** (Maeda T, 1996)(Van Eyken P, 1988) as well as the staining with a **pancytokeratin cocktail (AE1/AE3)**, that usually produces negative or weak staining, are less frequently used (Goodman ZD, 2007).

**CD34** is present and demonstrable in endothelial cells of large blood vessels and most capillary beds throughout the body, with the exception of normal hepatic sinusoidal endothelium. The trabeculae of hepatocellular carcinoma are surrounded by endothelial cells which phenotypically resemble capillary endothelium and are usually positive for CD34. On the contrary, normal hepatic sinusoidal endothelium and benign hepatocellular lesions typically have CD34 positive sinusoids only in areas that receive increased arterial

blood: cirrhotic nodules tend to be positive only around the periphery of regenerative nodules and focal nodular hyperplasia may show immunoreactivity for CD34 restricted to the sinusoids bordering fibrous septa (Goodman ZD, 2007). A diffuse, regular CD34 positivity of sinusoids can be helpful in distinguishing a cirrhotic nodule from a well-differentiated hepatocellular carcinoma. The staining for CD34 in hepatocellular adenomas is variable: this means that caution should be taken in the interpretation of CD34 reactivity in well differentiated tumor lesions, because a positive stain does not necessarily indicate malignancy (Varma V 2004)(Gouysse G, 2004).

**CD68** is a transmembrane glycoprotein located within lysosomes and endosomes. Thus, macrophages as well as other cell types rich in lysosomes/endosomes are CD68-positive (Ross HM, 2011). The staining pattern of CD68 was explored in the epithelial cells of both fibrolamellar and typical hepatocellular carcinomas In the romer, CD68 reactivity was found in the majority of cases, whereas it was absent in classical hepatocellular carcinoma (Ross HM, 2011).

## 4. Differential diagnosis

The diagnosis of hepatocellular carcinoma is sometimes very straightforward, not requiring anything other than hematoxylin eosin stained sections, whereas in most cases it requires the proper evaluation of several fine histological features, the reticulin framework, and the type of neo-vascularization. Two types of benign lesions enter into the differential diagnosis of hepatocellular carcinoma: hepatocellular adenoma in a noncirrhotic liver, and dysplastic nodules in the setting of cirrhosis (Ishak KG, 2001)(International Working Party, 1995)(Theise ND, 2002). The most frequent problem is distinguishing poorly differentiated hepatocellular carcinoma from other malignancies, especially metastases but also from poorly differentiated cholangiocarcinoma. A large cell carcinoma with eosinophilic cytoplasm, prominent nuclei and nucleoli may well be hepatocellular but that diagnosis should not be made without definite evidence of hepatocellular differentiation. Bile, recognizable canaliculi on haematoxylin-eosin or CEA stain, a positive stain for alphafetoprotein, a granular staining pattern with HepPar-1, or a trabecular growth pattern allow a diagnosis of hepatocellular origin of tumor cells with more or less certainty. However, the very rare cases of liver metastasis from a gastrointestinal adenocarcinoma with hepatoid features should be excluded, particularly when no other evidence of hepatocellular differentiation is found (Porcell , 2000). The tumor could be metastatic than primary in the liver, especially in the absence of cirrhosis. Cholangiocarcinomas and metastatic adenocarcinomas typically have a desmoplastic stroma, in contrast to hepatocellular carcinoma. A tumor with abundant stroma is almost always an adenocarcinoma, with the exception of the rare fibrolamellar type and even rarer scirrhous type of hepatocellular carcinoma (Craig JR, 1980)(Soreide O, 1986). Other metastatic malignant tumors may also be confused with hepatocellular carcinoma, in particular those characterized by large tumor cells with abundant cytoplasm, including melanomas (especially amelanotic) carcinoids, some poorly differentiated neuroendocrine tumors and renal cell carcinomas.

### 4.1 Hepatocellular carcinoma versus hepatocellular adenoma

Hepatocellular adenoma typically develops in women in the reproductive age group, and is often associated with oral contraceptive steroid use. Microscopically, they are composed of benign-looking hepatocytes arranged in sheets and cords without acinar architecture.

Adenoma cells are usually larger and paler than nontumor hepatocytes of the surrounding liver, due to increased cytoplasmic glycogen and/or fat. The nuclei of the tumor cells are typically uniform and regular, the nuclear/cytoplasmic ratio is low, and mitoses are almost never seen (Tab. 1). A well-developed reticulin framework is usually present in the tumor (Goodman ZD, 2007). The sinusoids, with flattened endothelial lining cells, are usually compressed, thus contributing to the sheet-like appearance. Sometimes the sinusoids are dilated, a finding which can be mistaken for peliosis hepatis. Bile ducts are not found in hepatocellular adenoma, but ductules and progenitor cells may be present. The presence of dilated sinusoids and ductules has caused some tumors to be classified as a telangiectatic variant of focal nodular hyperplasia, but molecular studies have shown these to be a variant of hepatocellular adenoma (Paradis V, 2004).

### 4.2 Hepatocellular carcinoma versus dysplastic nodule

Dysplastic nodule is the term used for a benign lesion that can be confused with hepatocellular carcinoma (International Working Party, 1995)(Theise ND, 2002)(Hytiroglou P, 2004). These are nodules in a cirrhotic liver that are macroscopically distinct from the surrounding cirrhotic nodules. They are usually larger than surrounding nodules and may be detected by imaging studies. They may also differ in colour or texture and may bulge from the surface of the liver (Goodman ZD, 2007). Distinguishing a well-differentiated hepatocellular carcinoma from a regenerative nodule or from a dysplastic nodule may be very difficult, particularly in small needle aspiration or core biopsies (Libbrecht L 2006). Histological examination is required to distinguish a dysplastic nodule from a small hepatocellular carcinoma (Tab. 2), and they are further classified as low-grade or high-grade, based on morphologic features (Goodman ZD, 2007).

**Low-grade dysplastic nodules**

Low-grade dysplastic nodules are sometimes vaguely nodular but are often distinct from the surrounding cirrhotic liver because of the presence of peripheral fibrous scar. This is not a true capsule, but rather condensation of scarring as is seen around all cirrhotic nodules (ICGHN, 2009). Architectural changes beyond clearly regenerative features are not present; these lesions do not contain pseudoglands or markedly thickened trabeculae. Unpaired arteries and portal areas are sometimes present in small numbers (Park YN, 1998). Nodule-in-nodule lesions are not present. Low-grade dysplastic nodules are composed of liver cells that are minimally abnormal, showing mild increase in cell density with a monotonous pattern, and they have no cytologic atypia, though they may have large cell change, formerly referred to as large cell dysplasia and or atipia (Anthony PP, 1973). The nuclear/cytoplasmic ratio is normal or slightly increased and there are no mitoses. The large cell atipia is charcterized by slightly nuclear pleomorfism (aneuploidia), multinucleation, abundant cytoplasm, mixed with normal hepatocyte. Steatosis may be present and there may be Mallory bodies (Goodman ZD, 2007). Low-grade dysplastic nodules may have diffuse siderosis or diffusely increased copper retention (ICGHN, 2009). Iron may be increased or decreased compared to the surrounding cirrhotic liver (Goodman ZD, 2007). Among members of the consensus panels ICGHN, 2009 there was no serious difficulty in differentiating Low-grade dysplastic nodules from early hepatocellular carcinoma. At the opposite end of the spectrum, distinction between Low-grade dysplastic nodules and large regenerative nodules was often found to be difficult or impossible. Therefore, there

is currently consensus that distinction between these two diagnostic categories cannot be made confidently by morphology alone and remains a task for the future. Fortunately, this distinction does not appear to have significant practical consequences at present (ICGHN, 2009).

**High-grade dysplastic nodules**

High-grade dysplastic nodules may be distinctly or vaguely nodular in the background of cirrhosis, although they also lack a true capsule, similar to low-grade dysplastic nodules; however, they are more likely to show a vaguely nodular pattern than low-grade dysplastic nodules. An high-grade dysplastic nodules is defined as having architectural and/or cytologic atypia, but the atypia is insufficient for a diagnosis of hepatocellular carcinoma. These lesions most often show increased cell density, sometimes more than 2 times higher than the surrounding nontumoral liver, often with an irregular trabecular pattern (ICGHN, 2009). Small cell change, also known as small cell dysplasia or atipia is the most frequently seen form of cytologic atypia in high-grade dysplastic nodules (Watanabe S, 1983). Small cell atipia refers to clusters of cells with features that suggest increased cellular proliferation. Hepatocytes show small or regular size, with hypercromic irregular nuclei, nuclear groove, anphophilic cytoplasm, nodular pattern, decreased reticolin small cell change. Plates more than two cells thick, pseudogland formation, cytoplasmic basophilia, higher nuclear/cytoplasmic ratio, nuclear hyperchromasia or an irregular nuclear contour are the most suggestive diagnostic elements (Goodman ZD, 2007). These features often confined to one or more foci within the nodule, giving the appearance 'nodule-in-nodule' formation (Goodman ZD 2007). This form of atypia may also occur in small hepatocellular foci outside of high-grade dysplastic nodules; the term dysplastic focus (International Working Party, 1995) may be appropriately used for such lesions. Large cell change may or may not be present in high-grade dysplastic nodules. Unpaired arteries are found in most lesions, but usually not in great numbers. A nodule-in-nodule appearance is occasionally found in high-grade dysplastic nodules, and subnodules often have a higher labeling index of proliferating cell nuclear antigen or Ki-67 than that of high-grade dysplastic nodules parenchyma. When a nodule with largely high-grade dysplastic features contains a subnodule of hepatocellular carcinoma, the subnodule of hepatocellular carcinoma is usually well-differentiated with a well-defined margin. The diagnostic discrepancy between high-grade dysplastic nodules and early hepatocellular carcinoma was frequent at the first consensus meeting, but it remarkably improved at the second meeting, due to the recognition of stromal invasion as a diagnostic criterion for the differentiation of high-grade dysplastic nodules from early hepatocellular carcinoma (ICGHN, 2009). If areas of questionable invasion are present, immunostaining for keratins 7 or 19 may be useful; if such stainings demonstrate a ductular reaction, the focus is considered a pseudoinvasion and does not warrant a diagnosis of hepatocellular carcinoma (Park YN, 2007). It is generally accepted that high-grade dysplastic nodule are precursors of hepatocellular carcinoma based on several lines of evidence, including morphologic features intermediate between low grade nodules and hepatocellular carcinoma, the presence of foci of hepatocellular carcinoma in otherwise high-grade dysplastic nodules, and follow-up showing progression to malignancy in a few cases. In some cases in clinical practice, it may be impossible to distinguish a high-grade dysplastic nodule from a well differentiated hepatocellular carcinoma, especially in needle biopsies. The current ability to increase the survival of patients with hepatocellular carcinoma relies upon the surveillance of cirrhotic patients. Surveillance allows hepatocellular carcinoma

precursors (dysplastic nodules) and malignant tumors to be recognized at an earlier stage making cure possible. Radiology plays a major role in hepatocellular carcinoma diagnosis because hepatocellular carcinoma is characterized by neoarterial vascularisation with a typical imaging pattern. Current international guidelines have restricted the use of the liver biopsy to the characterization of hepatocellular nodules which remain diagnostically equivocal after imaging. Thus pathologists are today facing very challenging and often well differentiated lesions, leading to difficulties in distinguishing high grade dysplasia and well differentiated hepatocellular carcinoma. In this scenario novel concepts obtained through international consensus have been proposed with emphasis on hepatocellular carcinoma of small size (up to 2 cm) which includes 2 distinct types, the early and progressed hepatocellular carcinoma (Roncalli M, 2011). High nuclear/cytoplasmic ratio of tumor cells is the most reliable sign of malignancy, even when the nuclei are not atypical, and this feathures, in combination with trabecular or pseudoglandular growth patterns distinguishes well-differentiated hepatocellular carcinoma from benign hepatocellular lesions (Goodman ZD, 2007). One of the main features used in this distinction is stromal invasion, which is usually not present in needle biopsy material (Park YN, 2007). Sampling errors in the biopsies may significantly influence the final diagnosis. It is indeed these small and limited tissue samples that pathologists rely upon, to make real diagnoses. The differential diagnosis of small, well-differentiated hepatocellular nodules mainly rests on fine histological criteria which can be difficult to adequately evaluate on tiny samples (Goodman ZD, 2007).

**Early or small well-differentiated hepatocellular carcinoma**

Early hepatocellular are vaguely nodular and are characterized by various combinations of the following major histologic features (Hytiroglou P, 2004)(Kojiro M, 2006)(Hytiroglou P, 2007):

1. increased cell density more than 2 times that of the surrounding tissue, with an increased nuclear/cytoplasm ratio and irregular thin-trabecular pattern;
2. varying numbers of portal tracts within the nodule (intratumoral portal tracts);
3. pseudoglandular pattern;
4. diffuse fatty change; and
5. varying numbers of unpaired arteries.

Among these features, diffuse fatty change is observed in approximately 40% of cases (Kutami R, 2000). The characteristic features of early hepatocellular carcinoma are sometimes seen in larger tumors. Including well-differentiated tumors that measure over 2 cm and thus do not qualify for the designation of small hepatocellular carcinoma set forth by the IWP. The prevalence of fatty change decreases along with increasing tumor size; therefore, fatty change is uncommon in tumors larger than 3 cm. Fatty change is also uncommon in moderately differentiated hepatocellular carcinomas. Any of the features listed above may be diffuse throughout the lesion or may be restricted to an expansile subnodule (nodule-in-nodule). Most importantly, because all of these features may also be found in high dysplastic nodules, it is important to note that stromal invasion remains most helpful in differentiating early hepatocellular carcinoma from high dysplastic nodules (ICGHN, 2009).

Pathologists today are asked to provide timely and conclusive diagnostic reports for the management and therapy of radiologically equivocal hepatocellular nodules found in small

biopsy samples. Although the traditional hematoxylin-eosin-based morphology remains the milestone, integration with biological information is required to make biopsy interpretation more objective and reproducible. To support the morphological criteria, additional and more objective criteria of malignancy, such as stromal invasion and the composite expression of a number of tissue biomarkers (translated to clinical practice from expression studies of human hepatocarcinogenesis (Chuma M, 2007)(Paradis V, 2003)(Nam SW, 2005)(Llovet JM, 2006)(Wurmbach E, 2007)(Di Tommaso L, 2007), have been proposed. Some have already been validated, as recently emphasized by Roskams and Kojiro (Roskams T, 2010), and their use in clinical practice has been suggested in the recent update of the AASLD practice guidelines (Bruix J, 2011). Indeed, the use of these tools can make pathologists, even those not specialized in liver pathology and in the diagnosis of liver tumor, more confident in the fine diagnostics of this challenging field. This is particularly true for small hepatocellular carcinoma, which is the most curable form and is particularly difficult to recognize with imaging. Non invasive imaging techniques were successful in 1- to 2 cm hepatocellular carcinoma detection in patients with cirrhosis in only 33% of cases (Forner A, 2008). In these cases the application of a panel of putative markers, such as glypican3, HSP70 and GS was thought to help to address the diagnostic challenge (Di Tommasi 2007). Hepatocelluar carcinoma is supported by the immunoreactivity of at least 2 markers while lack of immunoreactivity does not conclusively rule out malignancy. When morphological features suggest a non-malignant nodule an immunonegative profile is expected. In these cases, the staining of 2 markers should prompt reconsideration of the diagnosis. The panel may also be useful in clinically obvious but tangentially or minimally sampled tumors. It should be noted that in this setting the sparse peripheral neoplastic cells that might have been insufficient for a conclusive diagnosis of malignancy on hematoxylin eosin can easily be highlighted by the panel (Di Tommasi, 2009). The endothelial marker clathrin heavy chain (CHC) has been recently suggested to be diagnostically useful as well, particularly in combination with GPC3 (Seimiya M, 2008); its value was initially tested in surgical specimens (Seimiya M, 2008), and really also in liver biopsy samples (Di Tommasi 2011). A homogeneous series of small hepatocellular carcinoma no more then 2 cm in diameter and, for comparison, non small hepatocellular carcinomas sampled by a fine-needle approach (20-21 gauge) were immunostained for CHC with the aim of determining whether the addition of a novel marker (CHC) to the previously validated panel could increase the panel's diagnostic accuracy in the detection of small hepatocellular carcinoma (Di Tommasi 2011). The performance of the 4 markers' panel was superior to the performance of the same panel without CHC (the 3 markers' panel). With staining at least two markers, the accuracy was 97% and 84.3% in non small and small hepatocellular carcinomas, respectively, superior to the accuracy of the panel without the addition of CHC (86% and 76.9%, respectively). For small hepatocellular carcinomas, the addition of CHC to the panel consistently increased the sensitivity from 46.8% to 63.8%. Interestingly enough, for non small hepatocellular carcinomas, in cases in which the liver nodule was sampled with a 20 to 21 gauge needle, the accuracy of the novel panel (97%) was higher than the the previously reported (78.4%) with a 3M panel in an analogous hepatocellular carcinoma series sampled with 16- to 18-gauge needles. This means that the addition of CHC not only counterbalances the putative loss of sensitivity of thinner core materials but also increases the diagnostic accuracy. Although the use of a 4 markers' panel is more elaborate and time-consuming for pathologists, the unitary cost of an additional immunoreaction to the panel is much less expensive than confirmatory additional imaging (Sangiovanni A, 2010) or repeat

liver biopsies. When hepatocellular carcinoma series were dissected into subpopulations according not only to size but also to grading (G1 versus G2/G3), the panel accuracy remained excellent and greater than 90% for G2/G3 hepatocellular carcinomas, regardless of the size. This datum confirms that the performance of the 4 markers' panel is optimal when tumor differentiation is compromised; in other words, the individual markers of the panel cooperatively stain hepatocellular carcinomas that have progressed. Unfortunately, these are cases for which the pathological diagnosis can be rendered on morphological grounds without the use of staining beyond hematoxylin eosin. Interestingly, although the tumor size was not an issue in G2/G3 hepatocellular carcinomas, it was a major issue in well-differentiated (G1) hepatocellular carcinomas. Indeed, in this hepatocellular carcinoma group, which is the most difficult to evaluate in routine clinical practice, the accuracy of the panel was still excellent in non small G1 hepatocellular carcinomas (93.9%) but dropped to 67.4% in small G1 hepatocellular carcinomas. In the latter, the sensitivity for hepatocellular carcinoma detection was 50%, with 100% specificity, and the performance of the 4 markers' panel was much better than that of the 3 markers' panel. In addition, we noticed that a consistent fraction of these tumors showed negative staining (6/30, 20%) or one marker only (9/30, 30%). The most likely (though speculative) explanation for these conflicting results is that G1 hepatocellular carcinomas greater than 2 cm and G1 hepatocellular carcinomas smaller than 2 cm are not the same disease. An international agreement between Eastern and Western pathologists has recently been obtained for a new hepatocellular carcinoma entity: very well-differentiated, no more then 2 cm, hepatocellular carcinoma (which is also called very early hepatocellular carcinoma) (International Consensus Group for Hepatocellular Neoplasia, 2009). This is the earliest described and well-differentiated form of hepatocellular carcinoma and is likely the morphological link between high grade dysplastic nodule and hepatocellular carcinoma that has progressed. Very early hepatocellular carcinoma type is very difficult to recognize on imaging because of the immature vascular supply. A consistent fraction of the small G1 hepatocellular carcinoma cases likely belong to this very early type. The phenotypic profile of these cases is clearly distinct from that of other hepatocellular carcinomas of the present series, and this provides indirect proof of an earlier disease. Indeed, the small G1 hepatocellular carcinomas were less likely to be stained with the combination of the panel markers, their profile being intermediate between dysplasia (usually not staining) and hepatocellular carcinoma that has progressed (mostly staining). It is, therefore, reasonable to assume that when an hepatocellular carcinoma is just born, its phenotypic profile is not yet settled (e.g., the vascular support), and these markers are individually and progressively acquired and detectable. The most represented marker in small G1 hepatocellular carcinomas seemed to be CHC (58.8%), followed by GS (41.2%), HSP70 (17.6%), and GPC3 (11.8%). This means that in small G1 hepatocellular carcinomas, CHC is the most overexpressed marker. Thus, its evaluation, particularly in tumor core biopsy samples, is important, needs attention, and requires preliminary individual training. In particular, as for all the other markers under study, its staining should decorate putative malignant hepatocytes, and should appear as antigen overexpression in comparison with surrounding, adjacent nonneoplastic parenchymal cells. The prospective evaluation of nodules that remain diagnostically uncertain after biopsy could be very valuable for assessing the diagnostic strength of the present panel. Clearly, the search for additional and early markers has just started and is far less than completed. In conclusion, in core biopsy specimens of hepatocellular carcinomas sampled with a 20-to 21-gauge needle, the addition of CHC to a panel composed of GPC3,

HSP70, and GS increases the overall diagnostic accuracy in both small hepatocellular carcinomas (from 76.9% to 84.3%) and nonsmall hepatocellular carcinomas (from 86% to 97%), and there is an important gain in sensitivity in the detection of small hepatocellular carcinomas (from 46.8% to 63.8%). Absolute specificity was obtained only when two of the four markers were positive (regardless of which ones). Accuracy for hepatocellular carcinoma detection was not affected by the tumor size in G2/G3 hepatocellular carcinomas (>90%). In G1 hepatocellular carcinomas, tumor size played a major role in discriminating cases, with higher accuracy for nonsmall hepatocellular carcinomas (93.9%) and lower accuracy for small hepatocellular carcinomas (67.4%); likewise, the sensitivity was 88.2% for nonsmall hepatocellular carcinomas and 50% for small hepatocellular carcinomas. This results suggest that small G1 hepatocellular carcinomas include early tumors characterized by a relatively silent phenotype and the progressive acquisition of the markers is under study. The use of the present panel of markers supports the recognition of both small and nonsmall HCCs in the diagnostic pathology of challenging cases sampled by core biopsy (Di Tommasi 2011).

The combination of more than one putative marker of malignancy raises the overall accuracy. Glypican 3, HSP70 and GS have been found to be valuable in the differential diagnosis of hepatocellular carcinoma. A 3 markers' panel composed by HSP70, GPC3, and GS was demonstrated to be very useful in distinguishing between dysplastic and early malignant hepatocellular nodules arising in cirrhosis (Di Tommaso, 2007, 2009). When applying a panel of these three markers (GPC3, HSP70, and GS) to resected small lesions, the finding of any two positive markers had a sensitivity of 72% and a specificity of 100% to detect malignancy (Di Tommaso 2007, 2009). The diagnostic accuracy of this 3 markers' panel in liver biopsies of hepatocellular nodules has not been yet tested (ICGHN, 2009).

Immunohistochemistry should not be use as a diagnostic tool without consideration for morphology; on the contrary, it should always be carefully considered and dictated by morphology, and should serve as an ancillary tool to support a diagnostic conclusion. Hepatocellular carcinoma is often characterized by marked morphological and phonotypical intratumoral variability. Senes et al (2006) observed a striking variability in the degree of differentiation of the tumor cells among the 17 different sub-nodules detected in a case of hepatocellular carcinoma. Moreover, even the expression of the immunohistochemical markers utilized in this study varied greatly among different tumor regions (Senes et al 2006). As a consequence, in the case that, before the surgical resection, a needle biopsy had been performed, it was hypothesized that the interpretation of the bioptic core could have lead to different diagnoses, depending on the different tumor region sampled. This case underlines a previously unreported major role for sampling variability in the interpretation of needle biopsies and the possibility that, in clinical practice, when a very small fragment of the liver tumor is obtained by an ultrasound guided biopsy, immunoreactivity of the observed tumor cells could not sorely represent the distribution of tumor markers in the whole neoplasm, leading to sampling variability related diagnostic mistakes, not only for defining tumor grading but even in the differential diagnosis between primary and secondary liver tumor.

### 4.3 Hepatocarcinoma versus other malignant neoplasia

The differential diagnosis between hepatocellular carcinoma, cholangiocarcinoma and metastatic colorectal adenocarcinoma may be difficult when only based on morphology

(Terracciano , 2003). In fact, a subset of extrahepatic adenocarcinomas of different origin may show a solid "hepatoid" pattern virtually indistinguishable from hepatocellular carcinoma (Porcell , 2000). Similarly, metastases to the liver from various hepatoid variants of extra-hepatic neoplasms and other primary hepatic tumors, such as cholangiocarcinoma, may be mistaken for hepatocellular carcinoma (Varma V and Cohen C 2004). On the other hand, undifferentiated hepatocellular carcinoma may mimic poorly differentiated tumors of different origin, while its tubular and adenoid variants may be indistinguishable from cholangiocarcinoma or from metastatic colorectal adenocarcinoma. Furthermore, some of the unusual morphologic variants, including clear-cell, pleomorphic, and sarcomatoid variants, may be mistaken for metastases. The current literature shows that difficulties in histological typing of liver tumors, particularly in the differential diagnosis between hepatocellular carcinoma and cholangiocarcioma and metastases can be minimized by using immunohistochemistry (Varma V and Cohen C 2004)(Ma CK et al 1993). For that reason, in these cases, immunohistochemical analyses are often required (Stroescu , 2006). The panel of antibodies utilized to solve this differential diagnosis includes (tab. 2): cytokeratin 8-18 (Porcell , 2000)(Van Eyken P, 1988) Hep-Par1 (Leong AS , 1998) (Zimmerman , 2001) (Lugli A, 2004)(Minervini MI, 1997), glypican 3 (Yamauchi , 2005) (Libbrecht L 2006) (Zhu ZW et al 2001) (Capurro M et al 2003), cytokeratin 7 (Maeda , 1996) (Chu , 2000) (Durnez A et al 2006), cytokeratin 20 (Chu P, 2000)(Faa G , 1998), cytokeratin 19 (Maeda T, 1996)(Van Eyken P, 1988), CEA (Saad RS, 2004)(Goodman ZD, 2007) (Onofre AS , 2007) (Lau SK , 2002) and Alpha-fetoprotein (Onofre AS , 2007) (Lau SK , 2002) (Zhou L, 2006)(Wang L 2006). Among the numerous diagnostic immunohistochemical markers studied, alphafetoprotein (Wang L 2006), cytokeratin 7 (Durnez A et al 2006), cytokeratin 20 (Chu P et al 2000), cytokeratin 19 (Van Eyken P et al 1988), Hep-Par-1 (Zimmerman RL et al 2001)(Siddiqui MT et al 2002) and glypican 3 (Libbrecht L 2006) (Zhu ZW et al 2001) (Capurro M et al 2003) have been found to be the most valuable in the differential diagnosis between hepatocellular carcinoma and metastatic tumors. Immunoreactivity of tumour cells for cytokeratin 8-18, Hep-Par 1 and glypican 3 is considered suggestive of hepatocellular carcinoma; a diffuse immunoreactivity for cytokeratin 7 and cytokeratin 19 favours the diagnosis of cholangiocarcinoma; diffuse positivity for cytokeratin 20 and negativity for cytokeratin 7 are normally associated with metastatic colorectal adenocarcinoma. The sensitivity and specificity of the monoclonal antibody Hep-Par-1 for hepatocellular carcinoma are considered very high; as a consequence, the usefulness of this marker in the differential diagnosis of hepatic tumors is widely accepted (Chu PG et al 2002)(Saad RS et al 2004), although it stains normal hepatocytes as well. Moreover, expression profiling of primary hepatic tumors has demonstrated that glypican 3, a membrane-anchored heparan sulphate proteoglycan, is markedly expressed in hepatocellular carcinoma, particularly in well differentiated cases (Yamauchi N et al 2005). In spite of the availability of such armamentarium, daily experience shows that diagnostic mistakes can occur more frequently than generally expected. Indeed, some cases of hepatocellular carcinoma have been reported do not show immunoreactivity for Hep Par1 (Sugiki T et al 2004), nor for glypican 3 (Zhu ZW et al 2001). We reported a case of hepatocellular carcinoma with a peculiar immunohistochemical profile, characterized by the association of the typical immunoreactivity of hepatocellular carcinoma with a diffuse and strong positivity for cytokeratin 20 (Fanni D, 2009), generally considered typical of metastatic colorectal adenocarcinoma. The reason for this heterogeneity in immunoreactivity of hepatocellular carcinomas could be related to multiple factors: different etiology, variable degree of

differentiation, size of the bioptic core and sampling variability (Maharaj B et al 1998). The differential diagnosis of these lesions is often difficult, especially because of the scant material obtained by needle biopsy (Zhu ZW et al 2001).

## 5. Fibrolamellar carcinoma

Despite the distinctive clinical and histological features of fibrolamellar carcinoma, this entity can be a diagnostic challenge, because of lack of consistency of the diagnosis of fibrolamellar carcinomas due by pathologists (Ross HM, 2011). Even among experts, weak reproducibility was seen in the histological diagnosis of primary liver tumors with fibrous stroma in non-cirrhotic patients and a consensus diagnosis was achieved in only 32% of the cases (Malouf G, 2009). A large difference is seen in the average age of diagnosis for cases of fibrolamellar carcinoma published in the peer reviewed literature versus cases diagnosed in the SEER database, a difference most likely explained by inconsistencies in making the diagnosis of fibrolamellar carcinoma (El-Serag HB, 2004). Histologically, a common diagnostic pitfall is the overinterpretation of intratumoral fibrosis as being diagnostic of fibrolamellar carcinoma. In addition, another common reason for misdiagnosis is a misperception that most cases of primary liver carcinoma in children and young adults are fibrolamellar carcinomas, leading to overdiagnosis of fibrolamellar carcinoma in this age group, especially with tumors arising in livers without underlying disease (Ross HM, 2011). To improve the reproducibility of the histological diagnosis, over the past several years immunohistochemical stains that may be of assistance have been searched. The role for immunohistochemical markers as an aide in the diagnosis of fibrolamellar carcinomas has been previously explored but sought for additional markers among the currently used panel of antibodies available in most hospital laboratories (Vivekanandan P, 2004)(Klein WM, 2005). Fibrolamellar carcinomas show routinely strong immunoreactivity for CK7, while only about one third of typical hepatocellular carcinomas are CK7-positive (Klein WM, 2005)(Abdul-Al HM, 2010)(Ward SC, 2010). Further analysis of previously reported gene expression studies showed a modest increase in the expression of CD68 (Kannangai R, 2007). The immunohistochemical profile of fibrolamellar carcinomas was further extended to include CD68 staining, because of the routine availability of CD68 in most hospital laboratories: this approach can be helpful in routine surgical practice. CD68 staining among cases from four different institutions have been shown that metastatic tumors do not lose their CD68 positivity (Ross HM, 2011). A distinctive pattern of immunostaining for CD68 in fibrolamellar carcinomas was demonstrated. Nearly all cases showed a granular, stippled pattern, or a dot-like pattern of positivity. This staining pattern is highly sensitive for fibrolamellar carcinoma, but is not specific. In terms of its possible role in routine clinical use, given its high negative predictive value, a diagnosis of fibrolamellar carcinoma in a primary liver carcinoma that is CD68 negative should be strongly re-considered to ensure that fibrolamellar carcinoma is the appropriate diagnosis (Ross HM, 2011). In addition to the fibrolamellar carcinomas, this distinctive pattern was also seen in a minority of conventional hepatocellular carcinoma in both of hepatocellular carcinomas arising in cirrhotic and non-cirrhotic livers. Although not statistically significant, a larger percentage of conventional hepatocellular carcinomas that arose in non-cirrhotic livers showed CD68 staining. Whether this represents dysregulation of similar biological pathways is unclear. Also of note, CD68 positivity was seen adjacent to necrosis and CD68 staining in this context should be interpreted cautiously. Another point that bears emphasis is that none of the cases in the

control groups that were CD68 positive had the typical histological features of fibrolamellar carcinoma. Thus, combining the histological findings with CD68 would likely increase the specificity of CD68 staining for fibrolamellar carcinoma. The reproducibility of the histological diagnosis of fibrolamellar carcinoma can be substantially improved by careful attention to the full pattern of histological features, including large polygonal tumor cells with abundant eosinophilic cytoplasm, large vesiculated nuclei, large nucleoli, and lamellar fibrosis (Torbenson M, 2007). Moreover, most cases should be CK7 positive (Klein WM, 2005)(Abdul-Al HM, 2010)(Ward SC, 2010) as well as CD68 positive (Ross HM, 2011). In the absence of CK7 and/or CD68 positivity, a diagnosis of fibrolamellar carcinoma should be carefully re-considered. These findings also extend our understanding of the biology of

| Morphological feature | Hepatocellular carcinoma | Hepatocellular adenoma | Low grade dysplastic nodule | High grade dysplastic nodule |
|---|---|---|---|---|
| Cirrhosis background | 80% | Absent | 100% | 100% |
| Portal space | Variable | Absent | Present | Present |
| Bile duct | Variable | Absent | Present | Present |
| Isolated thick wall arteries | Present | Present | Rare | Rare |
| Architecture | Tick trabecular, pseudo-glandular or solid pattern | Sheet-like | Regenerative feature | Irregular trabecular pattern |
| Nodularity | Present | Not specific | Absent | Nodule in nodule |
| Reticolin | Absent | Present | Present | Increased and irregular |
| Cytological atipia | Present | Focal | Absent | Present |
| N/C, nuclear pleomorphism | Variable | Focal | Large cell dysplasia | Small cell dysplasia |
| Nucleoli | Present | Absent | Absent | Absent or small |
| Steatosis | Present | Present | Absent | Absent |
| Mitoses | Rare | Absent | Absent | Absent |
| Stromal and vascular invasion | Present | Absent | Absent | Absent |
| Ductular reaction (Ck7 e Ck19) | Variable | Absent | Present | Present |
| CD34 | Diffuse | Variable | Variable | Variable |
| Ki67 | High | Low | Low | Variable |

Table 1. The morphological features that differentiate the hepatocellular carcinoma from hepatocellular adenoma and from dysplastic nodules.

fibrolamellar carcinomas. Their oncocytic appearance has typically been attributed to cytoplasmic swelling by numerous packed mitochondria, as seen in electron microscopy studies. The CD68 positivity indicates that fibrolamellar carcinomas also have increased lysosomal or endosomal accumulations in their cytoplasm, which may suggest that abnormalities in endosomal/lysosomal trafficking are characteristic of fibrolamellar carcinomas. Overexpression of CD68 protein occurs at the level of both mRNA and protein (Ross HM, 2010). Of note, one previous study examined a case of fibrolamellar carcinoma and found it to be CD68-positive when using clone KP1 but not clone PG-M1 (Kaiserling E, 1995). They also reported that fibrolamellar carcinoma was weakly positive for Ki-M1P and 3A5, and negative for an anti-lysozyme antibody (Kaiserling E, 1995). These authors also reported that the non-neoplastic liver in a case of biliary atresia was CD68 positive (using clone KP1) and that, based on electron microscopy, immunoreactivity correlated with lysosome-like granules as well as electron dense structures representing probably bile components. Thus, some caution is likely warranted when staining a tumor rich in cytoplasmic bilirubin for CD68, as the bilirubin may be stored in lysosomes within tumor cells. In sum, CD68 immunostaining is a sensitive marker for fibrolamellar carcinoma that may be of use in routine diagnostic surgical pathology. In addition, it may be of use in research studies by helping properly classify cases of fibrolamellar carcinoma. This is of importance because it can help ensure a uniform biological entity, being investigated by different groups, as the incorporation of non-fibrolamellar carcinoma cases in such studies is unlikely to improve our understanding of the biology of this disease.

| Immunohistochemical markers | Hepatocellular carcinoma | Cholangiocarcinoma | Metastatic colorectal adenocarcinoma |
|---|---|---|---|
| Alpha-fetoprotein | + | - | - |
| pCEA | + | - | - |
| Hep-Par1 | + | - | - |
| Glypican 3 | + | - | - |
| Cytokeratin 8-18 | + | - | - |
| Cytokeratin 7 | - | + | - |
| Cytokeratin 19 | - | + | - |
| Cytokeratin 20 | - | -/+ | + |

Table 2. The panel of immunohistochemistry that helps to solve the differential diagnosis between the hepatocellular carcinoma from other malignant neoplasms

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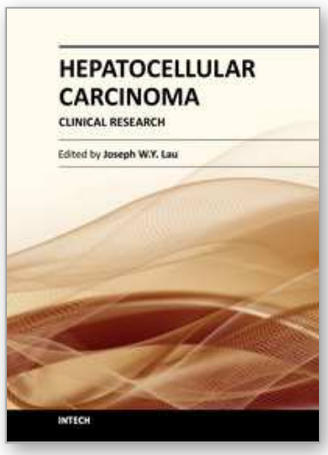
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This book covers the clinical aspects of hepatocellular carcinoma. This book is a compendium of papers written by experts from different parts of the world to present the most up-to-date knowledge on the clinical aspects of hepatocellular carcinoma. This book is divided into three sections: (I) Diagnosis / Differential Diagnosis; (II) Surgical Treatment; (III) Non-surgical Treatment. There are 19 chapters covering topics from novel diagnostic methods to hepatic lesions mimicking hepatocellular carcinoma, from laparoscopic liver resection to major hepatectomy without allogeneic blood transfusion, from molecular targeted therapy to transarterial radioembolization, and from local ablative therapy to regional therapy. This volume is an important contribution to the clinical management of patients with hepatocellular carcinoma. The intended readers of this book are clinicians who are interested in hepatocellular carcinoma, including hepatologists, liver surgeons, interventional and diagnostic radiologists, pathologists and epidemiologists. General surgeons, general physicians, trainees, hospital administrators, and instruments and drug manufacturers will also find this book useful as a reference.

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Phone: +86-21-62489820
Fax: +86-21-62489821

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