Recognising the clinically significant macrotrabecular massive variant of hepatocellular carcinoma

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Dear Sir,

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, and the key risk factors include infection with the hepatitis B virus (HBV), hepatitis C virus, metabolic syndrome and alcoholism. The overall prognosis of HCC is poor, with a 50%–70% rate of tumour recurrence despite interventions such as surgical resection and radiofrequency ablation. Recently, a novel histological subtype of HCC designated as the ‘macrotrabecular massive’ variant (MTM-HCC) was described by Calderaro et al.\(^1\) MTM-HCC is associated with poor survival and early recurrence, underscoring the need to recognise this clinically aggressive variant in order to better manage the disease.\(^2\)

Herein, we describe a case of an aggressive liver tumour showing morphological features of an MTM-HCC. However, its unusual immunophenotype, manifesting as a consistent negative staining for hepatocytic markers, prompted an extensive workup to exclude other primary liver and metastatic malignancies. Our case not only demonstrates the variable immunohistochemical features of MTM-HCC, but also highlights the importance of establishing a confident diagnosis of MTM-HCC on morphologic and clinical grounds.

A 65-year-old Chinese man with a history of chronic HBV infection showed an incidental solid and heterogeneous liver mass in the right lobe on ultrasonography. Serum alpha-fetoprotein (AFP) was 5.5 μg/L and carbonic anhydrase 19-9 (CA19-9) was 24.1 U/mL, which were within normal limits. Computed tomography (CT) imaging of the liver showed a 5.6-cm hypodense mass in segment 8/4A, with internal enhancement of the mass less than the adjacent liver parenchyma. The enhancement of the mass plateaued in the portal venous and delayed phases (Fig. 1a). No abdominal adenopathy or other masses were identified. As the radiologic features were not typical of a HCC, differentials such as cholangiocarcinoma and
metastasis were considered. No tumours were observed on oesophagastroduodenoscopy (OGD) and colonoscopy.

Primary resection of the liver mass was performed. Intraoperatively, a large subcapsular tumour in Segment 4A/8 was noted. No significant lymphadenopathy, cirrhosis or ascites was observed. Gross examination revealed two well-circumscribed, solid tumours with a firm, white cut surface and areas of necrosis, measuring 5.6 cm and 1 cm in their maximum extent, respectively. On histologic examination, both tumours showed neoplastic cells arranged in large solid nests and thick trabecular ribbons, with prominent central necrosis and intervening fine fibrous septae (Fig. 1b). The neoplastic cells were polygonal and epithelioid, with abundant eosinophilic cytoplasm and centrally placed round-to-ovoid nuclei with mild nuclear pleomorphism, fine chromatin pattern and occasional conspicuous nucleoli. Mitotic figures and apoptotic bodies were readily discerned (Fig. 1c). There was extensive vascular invasion (Fig. 1d) but no perineural invasion. Morphologically, the tumour was consistent with an MTM-HCC.

Immunohistochemical staining demonstrated negative HepPar-1 (Fig. 2a), Glypican-3, and Arginase 1 markers. There was strong diffuse positivity for CAM5.2 (Fig. 2b), glutamine synthase and Cytokeratin (CK) 20, and moderate positivity for CK7 and villin. Focal CK19 positivity was observed. CAIX showed moderate membranous positivity (Figs. 2c & d). CD34 was positive in sinusoidal endothelial cells, highlighting the trabecular pattern of the tumour (Fig. 2e). p63 and GATA3 were weakly positive. The following markers were negative: CK5/6, p40, BerEp4, CD10, CD56, synaptophysin, chromogranin, CDX2, MUC5AC, TTF1, 34BE12, Pax8, TdT, CD117, PSA, PSMA, WT1, calretinin, Sox10, melanA and desmin. The background liver showed features of chronic hepatitis B (Metavir stage 2/4). CCND1/FGF19 amplification was subsequently demonstrated on fluorescence in situ hybridisation (i.e. FISH, Fig. 2f) within tumour cells. The initial diagnosis was that of a poorly differentiated carcinoma,
with possibilities including a primary liver malignancy or metastases from gastrointestinal or urothelial tracts. With additional clinical workup including radiology, colonoscopy and OGD yielding no evidence of another primary malignancy, a diagnosis of MTM-HCC was finally established. Postoperatively, the patient opted for palliative treatment and was monitored with surveillance CT. Follow-up CT performed two months after the initial liver resection showed development of multiple larger and new hepatic masses in liver segments 2, 3, 5, 6 and 8, which suggested recurrence and progression of malignancy. Repeat surveillance CT imaging performed a month later showed further enlargement and new multiple hepatic masses, with interval invasion into the portal vein, and development of bilateral pleural effusion and moderate ascites. The patient eventually succumbed to his aggressive disease and died at four months after resection.

MTM-HCC is a novel histologic subtype of HCC that was first described by Calderaro et al.\(^1\) Its association with poor survival\(^{1,2}\) underscores the importance of recognising this entity so that clinicians can manage the patient appropriately. The estimated incidence of MTM-HCC is 10%–12% of all HCCs.\(^{1,2}\) It has been associated with HBV and high serum AFP level,\(^1\) the latter of which was interestingly lacking in our case. The imaging characteristics of MTM-HCC have not been described in the literature. In our case, the classic HCC features of arterial enhancement and washout in the venous and delayed phase on CT were not observed. Further studies on the imaging characteristics of MTM-HCC should be conducted to facilitate an early diagnosis of this aggressive subtype of HCC.

The histologic hallmark of MTM-HCC is a predominant (> 50%) macrotrabecular pattern consisting of trabeculae that are more than six cells thick. It also shows a significant association with vascular invasion.\(^1\) Our case of MTM-HCC showed negative staining for hepatocytic markers Glypican-3 and HepPar1. While there is no published data on the pattern of hepatocytic marker staining in MTM-HCC, the existence of HepPar1-negative HCC is well-
established and associated with specific HCC subtypes such as scirrhous HCC. Although Glypican-3 has been widely used as a highly sensitive marker for HCC, negative staining should not be considered as evidence to exclude HCC.\(^{(3)}\) In the current case, the tumour cells were positive for CK19 immunohistochemical staining, consistent with published literature that suggests that MTM-HCC has a CK19-positive progenitor phenotype\(^{(1)}\) that predicts early postoperative recurrence and increased invasiveness. CK20 positivity triggered the differential of a metastasis from the lower gastrointestinal tract; however, further investigations with immunohistochemical workup (CDX2, villin) and endoscopy showed negative results. An interesting feature in our case was the presence of membranous staining for CAIX, which is more commonly used by pathologists as a marker for clear cell renal cell carcinoma. CAIX is a known negative prognostic marker for HCC.\(^{(4)}\) One group of researchers has investigated the use of CAIX for diagnosis of MTM-HCC and reported a sensitivity of 48% and a specificity of 89%.\(^{(5)}\) Pathologists should thus be aware that HCC and its variants may express CAIX, to avoid misinterpretation of a metastatic renal carcinoma. Recently, ESM1, a vascular marker that highlights stromal endothelial cells lining macrotrabeculae, has been reported as a highly sensitive (97%) and highly specific (92%) marker of HCC.\(^{(5)}\) Where available, pathologists can consider using ESM1 as a diagnostic adjunct when faced with a case of HCC that is consistently negative for other hepatocytic markers.

Gene expression profiling studies in the past years have resulted in the establishment of several HCC transcriptomic classifications.\(^{(1)}\) The MTM-HCC subtype is associated with ATM mutations, angiogenesis activation, TP53 mutations and FGF19 amplifications,\(^{(1)}\) the latter of which was detected in our case. However, more studies are needed to investigate how the molecular classification can be translated into clinically relevant practices. In one of the larger series published about MTM-HCC to date, Ziol et al demonstrated that the MTM-HCC subtype is associated at baseline with known poor prognostic factors (tumour size, vascular
invasion, alpha-fetoprotein level and satellite nodules) and an independent predictor of early and overall recurrence. These findings underpin the aggressive clinical course of MTM-HCC, highlighting to clinicians the need to recognise this distinct group of tumour that warrants more personalised and aggressive treatment strategies than conventional HCCs do.

In summary, we presented a case of an aggressive and early recurring liver tumour with histomorphological features of MTM-HCC but unusual immunohistochemical features. While the immunohistochemical staining features were consistently negative for hepatocytic markers and even suggested the possibility of a metastasis, the absence of other primary malignancies detectable on further clinical workup and the convincing histomorphological features of the tumour provided strong support for a final diagnosis of MTM-HCC. Our case highlights the importance of using morphology as the primary guidance to assign pathological diagnoses to tumours and interpreting them in the context of relevant clinical and immunohistochemical findings.

Yours sincerely,

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REFERENCES


FIGURES

**Fig. 1** (a) CT image in delayed phase shows a hypodense mass in Segment 8/4a of the liver whose enhancement characteristics are not typical of a hepatocellular carcinoma. (b) Photomicrograph shows tumour cells arranged in large solid nests and thick trabecular ribbons (> 6 cells thick) with prominent central necrosis, with nests separated by intervening fine fibrous septae (H&E, × 10). (c) Photomicrograph shows large, polygonal tumour cells with abundant eosinophilic cytoplasm and central round to ovoid nuclei with granular chromatin, irregular nuclear contours and prominent nucleoli. Mitotic figures are readily discerned (H&E, × 40). (d) Photomicrograph shows extensive vascular invasion (H&E, × 4).
Fig. 2 Immunohistochemical and fluorescence in situ hybridisation (FISH) features of hepatocellular carcinoma. Photomicrographs of tumour cells show (a) negative staining for HepPar1 as well as Glypican-3 and Arginase 1 (not depicted); (b) positive staining for CAM5.2; (c & d) moderate membranous positivity for CAIX; (e) CD34 highlighting sinusoidal endothelial cells, accentuating the trabecular pattern of macrotrabecular-massive hepatocellular carcinoma; and (f) amplification of CCND1-FGF19 demonstrated by the FISH technique (green signal: FGF19 locus at 11q13.1, orange signal: centromere 11p).