Pathology (December 2019) **51(7)**, pp. 742–771

CORRESPONDENCE

Hepatocellular neoplasm of uncertain malignant potential associated with hepatic granulomas featuring p62-positive asteroid bodies

Sir,

Well-differentiated hepatocellular neoplasm of uncertain malignant potential (HUMP) describes a group of neoplastic liver lesions with atypical clinical and/or histological features that cannot be classified as either hepatocellular adenoma (HCA) or well differentiated hepatocellular carcinoma (HCC).[1] Here we present a case of HUMP with associated well-formed granulomas in the background liver parenchyma. Although the co-existence of HCA and hepatic granulomas has been shown before,[2] their occurrence in relation to a HUMP has not been previously described.

In addition, we report the serendipitous discovery that asteroid bodies in multinucleated giant cells of these epithelioid granulomas are positive for p62 (sequestosome-SQSTM1/A170/ZIP), a protein involved in autophagy regulation. This has not been reported previously and provides novel insights into the pathogenesis of asteroid bodies in granulomatous disease.

A 50-year-old woman presented with a large inhomogeneous mass in the left lobe of the liver seen on ultrasound to investigate a mildly elevated serum gamma-glutamyl transferase (GGT). Her body mass index (BMI) was 32 kg/m^2 and past medical history included type 2 diabetes mellitus, hypercholesterolaemia and hypertension. She reported taking norethisterone for 10 years, which was suspended on admission. None of her other medications have been associated with HCA, HCC or hepatic granulomas.

At presentation she was asymptomatic. All liver function tests were within the normal range, except for GGT of 127 IU/L. Alpha-fetoprotein, CA125 and carcinoembryonic antigen values were within normal limits and viral hepatitis and autoantibody screen were negative. Abdominal computed tomography and magnetic resonance imaging (Fig. 1A) demonstrated a 10 cm inhomogeneous exophytic hepatic lesion arising from the left lobe of the liver. This lesion was consistent with HCA but low grade malignancy could not be excluded. Therefore, a left lateral liver resection was performed (segments II and III).

The liver capsule was intact and smooth with a bulging tumour measuring 95 mm in greatest dimension and completely resected R0. On sections, the tumour was well-defined, non-encapsulated with cystic areas and haemorrhagic foci (Fig. 1B), extended very close to the capsule and was completely excised by 15 mm. There were no satellite tumours in the background non-cirrhotic liver but a well circumscribed, pale, 2 mm nodule was seen in segment III (Fig. 2A).

Histology showed a well differentiated hepatocellular neoplasm composed of sheets and irregular chords up to 2/3-cell thick with focal reticulin loss in two of the six tumour sections examined, extensive sinusoidal capillarisation (CD34 immunostain), large areas of haemorrhage, patchy necrosis and hyalinisation of the scant connective tissue stroma. There were unpaired arteries present in all six sections but no portal tracts, bile ducts or ductules. There was focal moderate nuclear atypia and scattered minute foci of small cell change in three of six sections, but no mitotic figures. There appeared to be no association between areas of microhaemorrhage and cytological atypia.

Occasional steatotic cells and foci of extramedullary haemopoiesis were noted (Fig. 1C–F). Many tumour cells expressed keratin 7 but there was no immunohistochemical expression of keratin 19 and glutamine synthetase and no aberrant nuclear or cytoplasmic β-catenin positivity. Further immunohistochemical analysis for HCA subtyping revealed retained liver fatty acid binding protein (LFABP) expression and absence of serum amyloid A (SAA) and C-reactive protein (CRP) immunostaining in the tumour. DNA analysis showed no mutations in exon 3 of the *CTNNB1* gene. The overall morphological appearances fulfilled the histological criteria of a well differentiated hepatocellular neoplasm of uncertain malignant potential (HUMP).[1]

The background liver showed mild to moderate steatosis and mild, non-specific, chronic inflammation without significant fibrosis (Fig. 2B,C). Numerous non-caseating epithelioid granulomas with multinucleated giant cells containing well defined asteroid bodies (Fig. 2C,D) were seen in portal tracts and in the lobules. The 2 mm nodule in segment III was composed of coalescing granulomas set in a fibrous stroma (Fig. 2B). There was no evidence of cholangiopathy and Ziehl–Neelsen (ZN)/modified ZN stains for mycobacteria were negative. A p62 immunostain performed to exclude the presence of early Mallory–Denk bodies, showed unexpected strong specific immunostaining of all asteroid bodies (Fig. 2E).

Extensive clinical investigation excluded sarcoidosis or infectious aetiology of the granulomatous hepatitis pointing to chronic use of oral contraceptive and/or a local response to the HUMP as the most likely causes. At 39 months post-surgery the patient remains well without any signs of recurrence or metastasis.

Hepatocellular adenoma (HCA) is a benign hepatic neoplasm which usually presents in young women (20–40 years). Recent European Association for the Study of the Liver guidelines recommend that small HCAs (<5 cm) presenting in women are managed conservatively and monitored with annual imaging.[3] Lesions larger than 5 cm, and those displaying exophytic protrusion, have a greater risk of haemorrhage so resection is recommended.[3,4]

Different subtypes of HCA have been described based on molecular classification: HNF1A-inactivated (40–50%), β-catenin exon 3 mutated (10–15%), β-catenin exon 7/8 mutated (5–10%), inflammatory (35–45%; some of these also have β-catenin mutations), sonic hedgehog HCA (5%), and unclassified HCA (<7%).[2] The molecular subtypes of HCA correlate more or less with specific morphological characteristics and can be diagnosed using immunohistochemistry for specific or surrogate markers.[3] The different HCA subtypes have varying prognosis and malignant potential, and their recognition could allow tailored management.[5] Recently, a new category of hepatocellular neoplasm

Print ISSN 0031-3025/Online ISSN 1465-3931

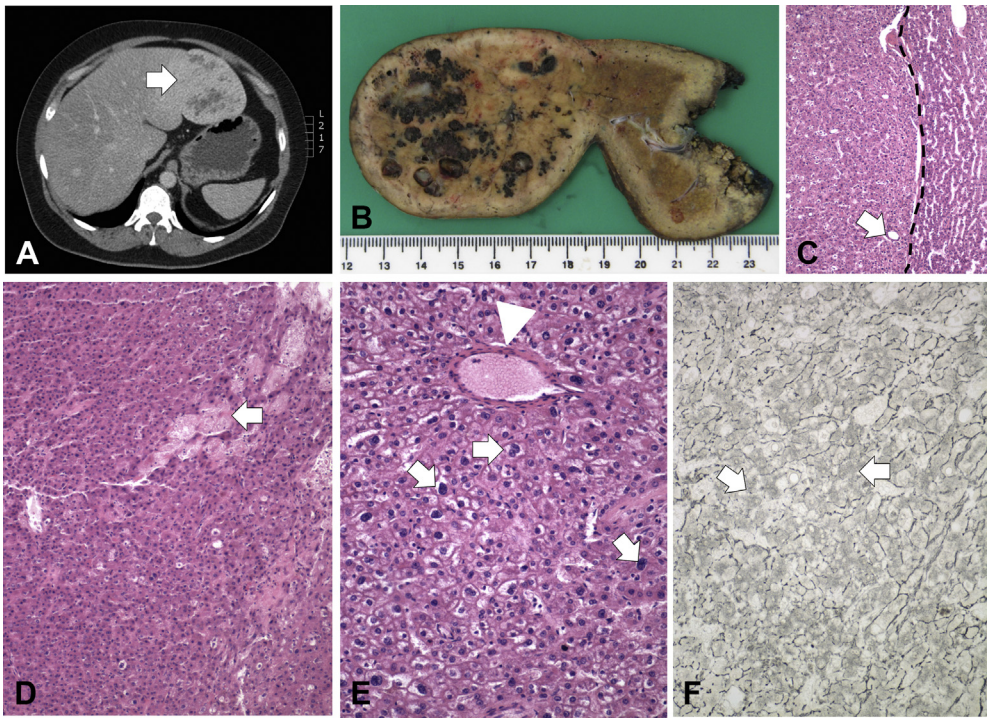

Fig. 1 (A) Contrast-enhanced CT image in transverse plane showing a large, well-defined, predominantly solid lesion in the left lobe of the liver (white arrow). Appearances are consistent with HCA, but low-grade malignancy could not be excluded. (B) Left lateral segmentectomy (II and III) specimen showing an intact smooth capsule covering a bulging mass. Grossly, sections show non-cirrhotic yellowish liver with a well-defined, non-encapsulated tan and cream tumour, with cystic spaces and foci of haemorrhage (C) Low magnification shows a hepatocellular neoplasm with pushing borders (dotted line) and unpaired arteries (white arrow) (H&E). (D) The neoplastic cells are arranged in trabeculae up to 3 cells thick with minimal intervening stroma and haemorrhagic areas (white arrow). No portal tracts, bile ducts or ductules are seen (H&E). (E) One of the many unpaired arteries (arrowhead) and mild nuclear pleomorphism and hyperchromasia (white arrows) (H&E). (F) Focal reticulin loss (white arrows) (reticulin stain).

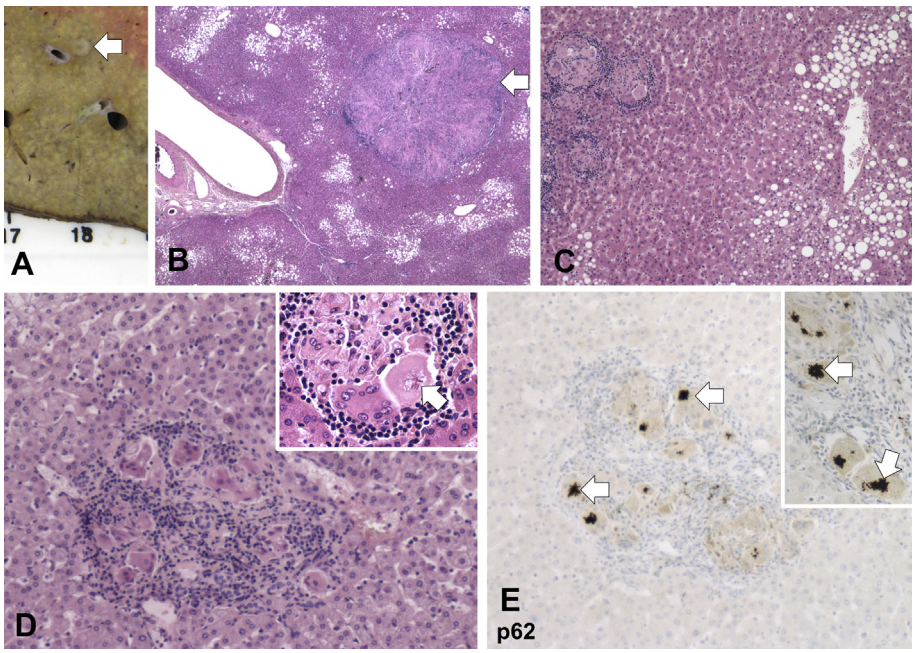

Fig. 2 (A) The background non-cirrhotic liver is yellowish and contains a 2 mm pale, circumscribed nodule (white arrow). (B) The nodule is composed of coalescing granulomas set in an inflamed fibrous stroma (white arrow). The surrounding liver shows mild steatosis (H&E). (C) Steatosis has a distinct zone 3 (centrilobular) accentuation. The granulomas (left) are non-caseating and contain epithelioid and multinucleated giant cells (H&E). (D) Higher magnification of an epithelioid non-caseating granuloma with numerous multinucleated giant cells (H&E), some of which contain well-formed asteroid bodies (inset, H&E higher magnification). (E) Immunohistochemistry for p62 shows strong specific positivity of all asteroid bodies (white arrows) and expected mild granular cytoplasmic positivity of some macrophages (DAB) (inset, p62-positive asteroid bodies in higher magnification).

has been proposed: well-differentiated hepatocellular neoplasm of uncertain malignant potential (HUMP).[1]

The term HUMP describes tumours which cannot be definitively classified as either HCA or well-differentiated hepatocellular carcinoma (HCC), replacing previous terms such as 'atypical hepatocellular neoplasm', 'atypical adenoma', and 'borderline HCA', which do not convey the need for close follow-up. A recent large study looking at 533 presumed HCA samples found that 7% of lesions were borderline between HCA and HCC following resection and histological analysis.[5] A detailed description of the proposed entities that may be classified as HUMP was published in 2014 by the International Liver Study Group 'Gnomes'.[1]

This case fits into the classification of HUMP for several reasons. Firstly, patient presentation was atypical for HCA, as she was 50 years old. Secondly, the hepatocellular lesion displayed histological atypia with focal reticulin loss, moderate nuclear atypia and small cell change. The diagnosis of HUMP is currently reliant on a combination of clinical and histopathological findings, and no specific radiological features of HUMP have been proposed thus far.[5] Indeed, in this case the radiological findings were consistent with HCA.[6] Regarding histological subtyping, the morphological features in our case were more consistent with inflammatory subtype but immunohistochemical findings (absence of SAA and CRP expression) were not confirmatory.

Interestingly, diffuse granulomas were present in the surrounding liver. Granulomatous change has been previously reported as a rare reaction to HCA.[2,7–9] A recently published case series reported five patients with HCA and associated hepatic granulomas. Four of five HCAs fell into the inflammatory subtype, leading the authors to hypothesise that chronic irritation and inflammatory stress could be the causative factor in granuloma formation.[2] Indeed, in the current case, morphological signs of inflammation were present in the surrounding liver.

Local granulomatous change has long been recognised as a rare consequence of malignant disease.[10] Specifically, the association of epithelioid granulomas and HCC has been reported.[10,11] Antigenic factors released by tumour cells can elicit immunological hypersensitivity reactions, resulting in granuloma formation.[10,11] As HUMP describes a neoplasm with certain histological features of well differentiated HCC, it could be that tumour factors released by HUMP are the causative agent in granuloma formation.

Alternatively, the granulomatous disease could have preceded the development of HUMP. Chronic inflammation caused by granulomas could provide the basis for neoplasm development. This theory that granulomas are the causative agent is not favoured, as previous cases have seen resolution of granulomatous hepatitis after the associated HCA has been removed.[9]

The multinucleated giant cells within the granulomas in the present case contained asteroid bodies which stained strongly positive for the stress protein p62/sequestosome-1. This was a serendipitous finding as p62 immunostaining was performed to visualise possible early MDBs in the background steatotic liver. The exact pathogenesis and composition of granuloma asteroid bodies is not fully understood, and no previous study has identified the presence of p62 within asteroid bodies. As the primary function of p62 is to label substrates for degradation by autosomes,[12] our hypothesis is that defects in autophagy within multinucleated giant cells prevent clearance of p62 labelled substrates. This then leads to substrate aggregation, which is eventually apparent as p62-positive asteroid body aggregates. Further research investigating the role of autophagy and p62 in asteroid body formation is required.

In conclusion, HUMP describes a group of hepatic neoplasms with uncertain malignant potential which histologically fall between HCA and well-differentiated HCC. Lack of recurrence or metastasis at 39 months post-surgery in this case supports a benign course for HUMP following resection. The finding of granulomas in this case fits with previous research showing granulomatous change surrounding HCA/HCC. The novel finding that asteroid bodies within these granulomas stain positive for p62 suggests that defects in autophagy may play a role in their development.

Acknowledgements: The authors thank Dr Chris Bellamy, Division of Pathology, University of Edinburgh, UK, and Dr Rosa Miquel, Institute of Liver Studies, King's College Hospital, London, UK, for facilitating immunohistochemistry for LFABP, SAA and CRP and for insightful comments on the case presented. The help of Mr John Moir in providing and interpreting the radiological figure in this report is acknowledged with thanks.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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DOI: https://doi.org/10.1016/j.pathol.2019.08.011

Rare case of spindle cell/ sclerosing rhabdomyosarcoma in adult liver

Sir,

Rhabdomyosarcoma (RMS) is a common soft tissue sarcoma in children that can infrequently present in adulthood.[1] Although alveolar and embryonal RMS are the most common (>80%) of all histological subtypes encountered, rare variants can occur. Seldom, RMS can involve solid organs. Herein, we present a case of spindle cell/sclerosing variant of RMS in an adult liver, first diagnosed on a partial hepatectomy specimen for suspected cholangiocarcinoma.

A 57-year-old Caucasian female with no significant past medical history presented with a 6-week history of shortness of breath, epigastric discomfort and fatigue. Magnetic resonance imaging with intravenous contrast identified a large (>18.0 cm) exophytic left hepatic lobe mass (Fig. 1A) elevating the left hemi-diaphragm and impinging on the heart, together with multiple omental nodules. Imaging findings were interpreted as concerning for intrahepatic cholangiocarcinoma with peritoneal carcinomatosis. The patient's blood work-up revealed mild anaemia (Hb 11.2 g/dL; range 12−15 g/dL) but clinical biochemistry results were within normal limits including serum tumour markers CA125, CA19.9 and CEA. The patient underwent partial hepatectomy, left hemi-diaphragm resection and omentectomy. Surgical specimen showed an 18.5 cm white-yellow, fleshy liver mass (Fig. 1B) adherent to the diaphragm. Histological examination demonstrated a spindle cell lesion composed of fascicles of cells with eosinophilic cytoplasm and elongated nuclei (Fig. 2A). Frequent strap cells with cross striations (Fig. 2B) and multiple areas of stromal hyalinisation/sclerosis with pseudovascular arrangement of tumour cells (Fig. 2C) were also noted. Omental nodules showed similar tumor histology.

On immunohistochemical studies, the neoplastic cells showed expression of Myo-D1 (strong and diffuse; Fig. 2D), desmin and myogenin (patchy) with no expression of cytokeratins, SOX-10, HMB-45 and Melan-A. Interphase fluorescent *in situ* hybridisation was negative for *FOXO1* gene rearrangement. The morphology and immunophenotypic features of this liver mass were diagnostic of RMS, with further categorisation into a spindle cell/sclerosing variant of rhabdomyosarcoma (SRMS), per the current World Health Organization (WHO) classification of soft tissue tumours.[2]

Four broad categories of RMS are recognised by the 2013 WHO classification, namely alveolar, embryonal, pleomorphic and spindle cell/sclerosing.[3] The 'spindle cell' and 'sclerosing' variants were separate entities individually described first by Cavazzana *et al*.[4] and Mentzel and Katenkamp,[5] respectively. SRMS is classified as a standalone variant in the current (2013) edition of the WHO classification system[3] after studies showed that both the spindle cell and sclerosing variants share similar clinical, histopathological, and molecular characteristics. SRMS comprises 5−10% of all RMS cases, with a predilection for the head and neck region, and rarely presents in adulthood.[2] At the time of diagnosis of our case (May 2018), SRMS had not been reported in the liver; however, recently Agaram *et al.*, described one case of RMS with pure spindle cell morphology within the liver.[6] In contrast, our case demonstrated both spindle cell and sclerosing morphologies that were intermixed. Embryonal and alveolar RMS are more common, and both variants have been previously reported in adult liver, albeit in rare case reports.

Histomorphology of SRMS can be characteristic as described above and as depicted in Fig. 2A−C, facilitating a histological diagnosis. Immunohistochemical studies that are particularly helpful in the diagnosis of SRMS include myogenin, Myo-D1, desmin and vimentin, all of which can show variable staining. Studies on SRMS have previously demonstrated that the neoplastic cells in all cases tend to express Myo-D1.[6,7] Diffuse and strong positivity for Myo-D1

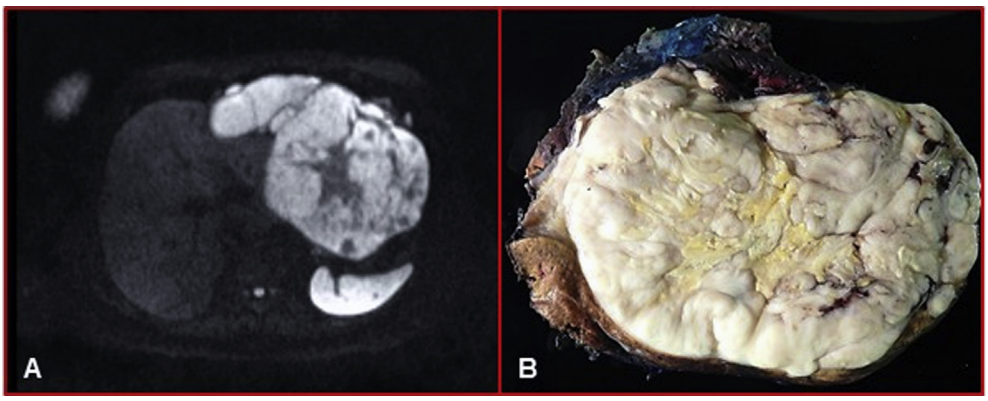

Fig. 1 (A) Abdominal magnetic resonance imaging with IV contrast showing a large mass in the left hepatic lobe. (B) Surgical specimen showing a well-defined fleshy mass with a thin rim of surrounding liver parenchyma.