HEPATIC MASS IN A FLAT COAT RETRIEVER

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Case presentation:

A 10 year old female neutered Flat Coat Retriever was presented to the Queen Mother Hospital for Animals, Royal Veterinary College, UK with a recent history of intermittent short episodes of weakness after exercise, lethargy and weight loss.

Biochemistry revealed moderate ALT elevation (338 U/L, reference interval [RI] 13-88) and marked ALP elevation (3470 U/L, RI 19-285), mild hypercholesterolemia (10.8 µmol/L, RI 3.3-8.9) and mild increase of urea (12.2 mmol/L, RI 3.0-9.1) with creatinine within RI. Haematology revealed mild lymphopenia only and blood smear examination was unremarkable.

Imaging identified a mass within the right side of the liver, which appeared iso- to hypoechoic on ultrasound and mixed attenuating with contrast enhancement on computed tomography (CT). The size of the mass was determined to be approximately 8x11 cm, occupying the right medial and lateral liver lobes with possible involvement of the caudate lobe.

Fine-needle aspirates were obtained from the hepatic mass and submitted for cytologic evaluation (Figures 1 to 4).

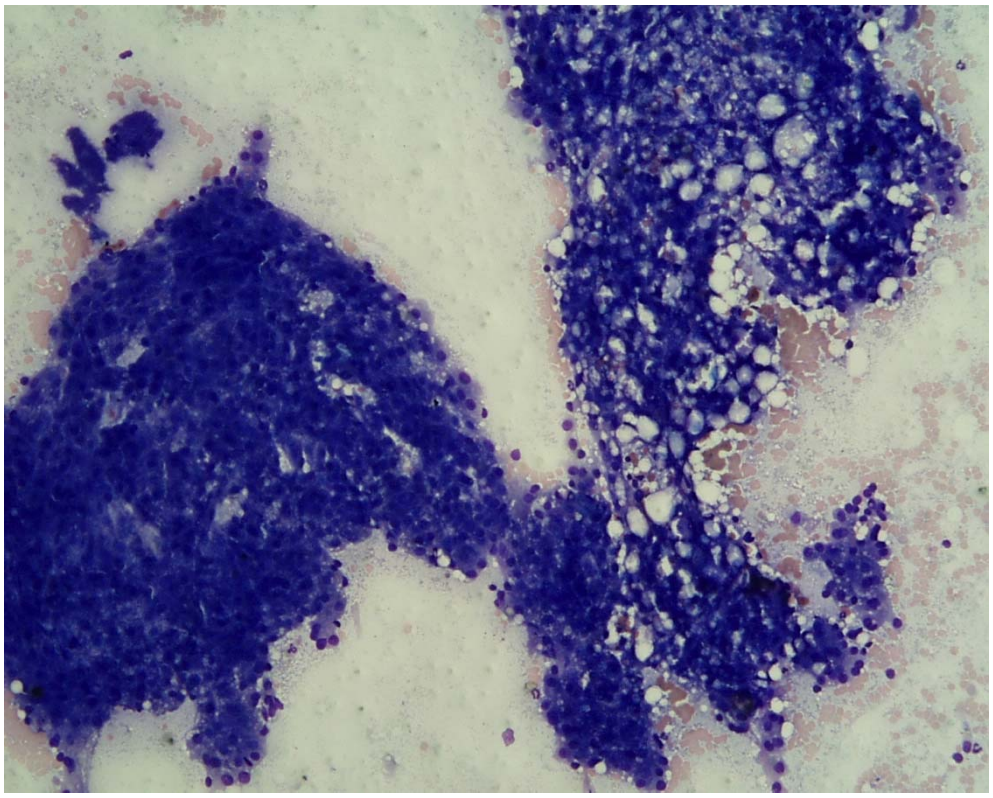

Figure 1: FNA from hepatic mass (100x Magnification, Modified Wright's)
Low power view shows a sheet which has more typical hepatocyte appearance (left) and one in which macrovesicular vacuolation predominates (right)

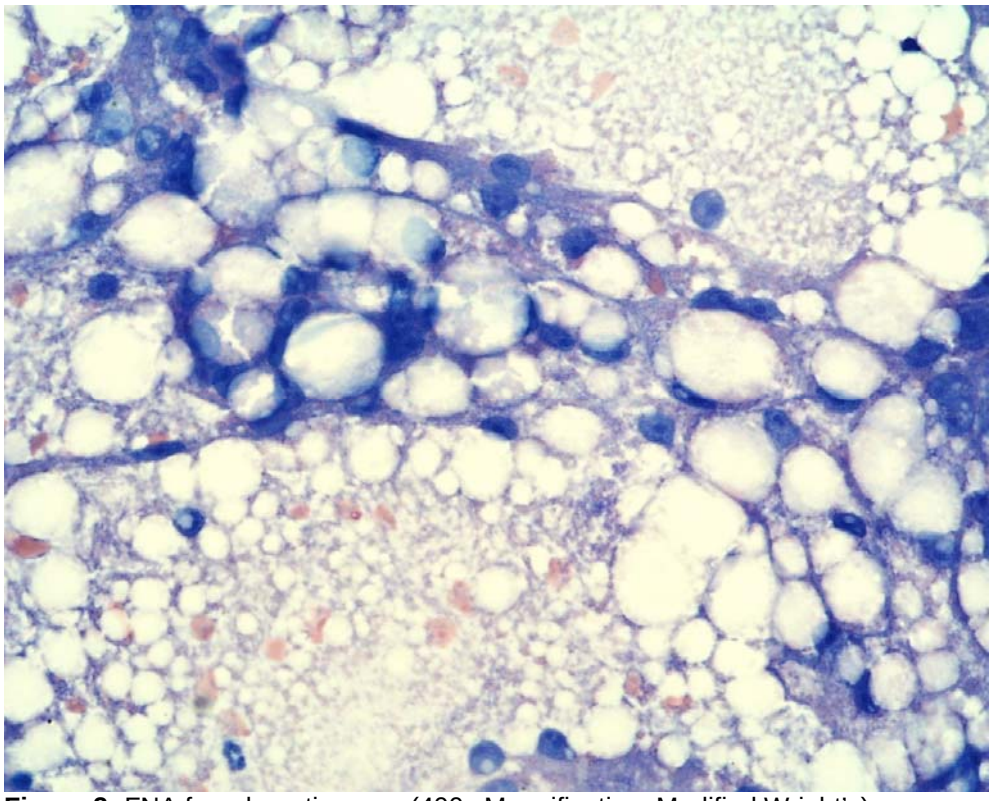

Figure 2: FNA from hepatic mass (400x Magnification, Modified Wright's)
Many cells have marginalised nuclei

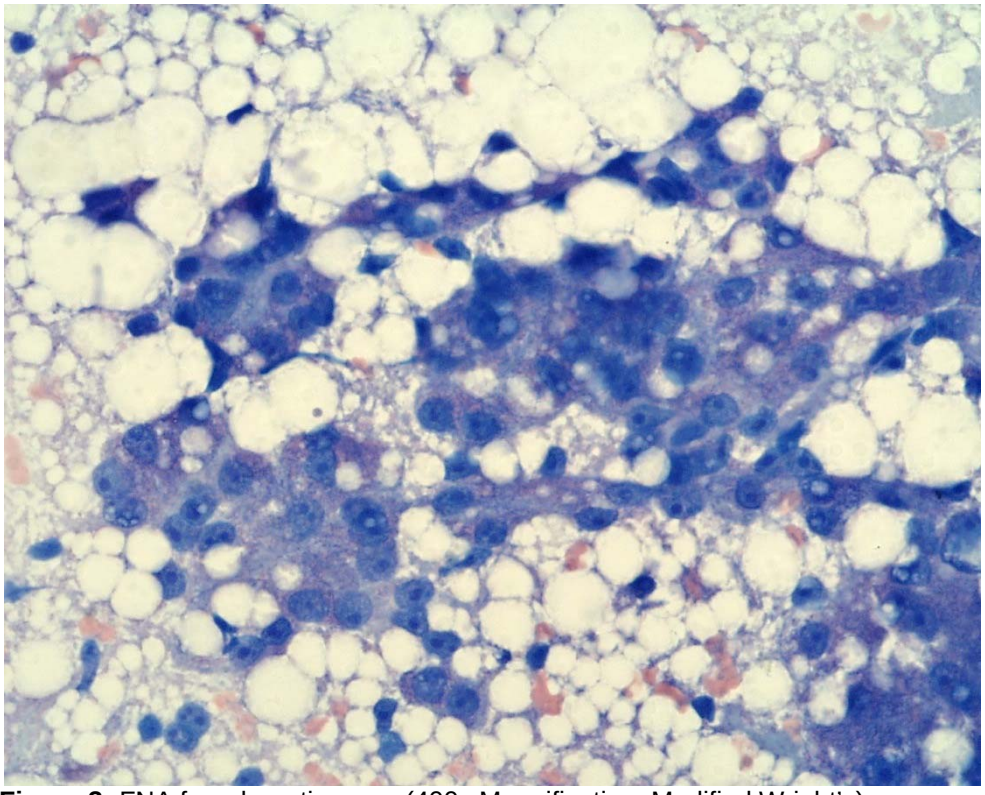

Figure 3: FNA from hepatic mass (400x Magnification, Modified Wright's)
Occasional cells appear trinucleated and occasionally multiple nucleoli are present.

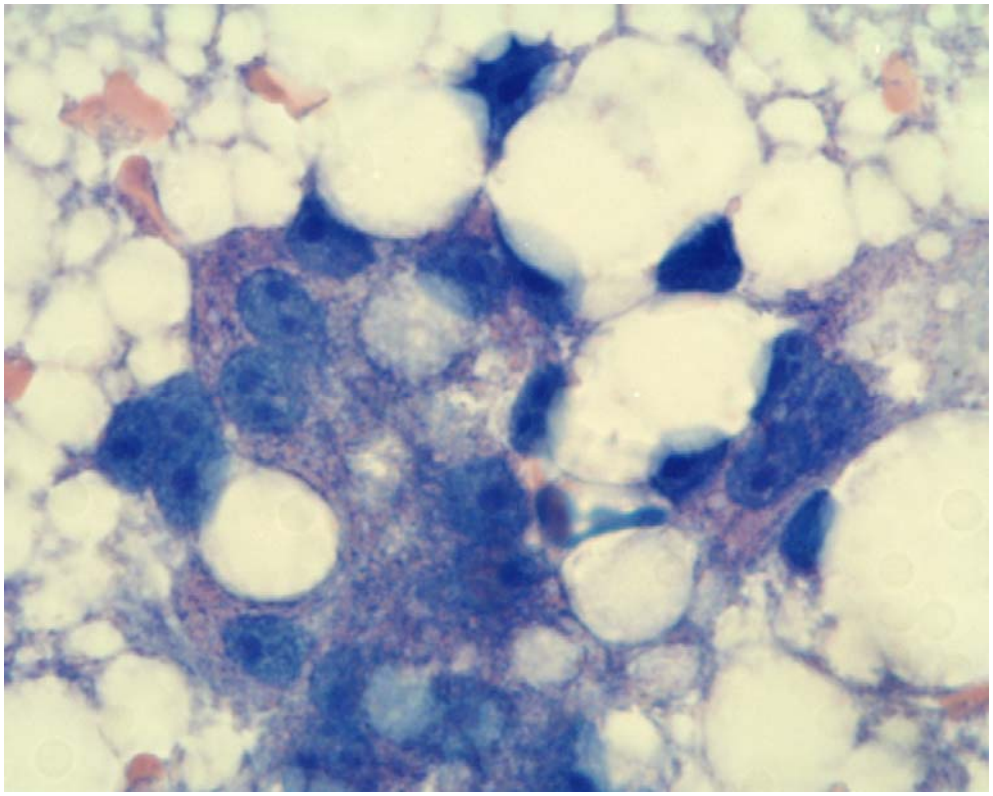

Figure 4: FNA from hepatic mass (1000x Magnification, Modified Wright's)
Occasional cells appear multinucleated and occasionally multiple nucleoli are present.

Cytologic description:
Smears were of high cellularity. Hepatocytes were noted in large sheets, frequently appearing crowded and disorganised. Nuclei were round to oval, and possessed finely stippled chromatin, with up to three prominent basophilic nucleoli. Cells had abundant to occasionally low amounts of granular amphophilic cytoplasm, occasionally more basophilic in appearance with variably extensive, often marked cytoplasmic macrovesicular change. The macrovesicular change was particularly prominent and affecting the majority of cells on one of the smears. In these areas, the cytoplasm frequently consisted of a single vacuole displacing the nucleus (signet-ring appearance). Occasional hepatocytes showed cytoplasmic rarefaction. Hepatocytes showed mild to moderate anisocytosis and moderate anisokaryosis with a mildly increased N:C ratio, frequent binucleation, occasional multinucleation, rarely with up to four nuclei, occasionally multiple nucleoli, occasional macronucleoli and rare nuclear moulding are present. Abundant lipid vacuoles were present in the background and amongst the sheets of cells.

Cytologic interpretation: Marked vacuolar hepatopathy with macrovesicular vacuolar change (lipid), concern for hepatocellular neoplasia.

Histopathologic description:
Tru-cut biopsies revealed sheets of neoplastic hepatocytes without discernible normal hepatic architecture lacking portal areas and with occasional hepatocellular rosettes. Approximately 70% of neoplastic cells had vacuolated cytoplasm, predominantly with single large vacuoles, less commonly multiple small vacuoles. Mild anisokaryosis, 1-2 nucleoli per nucleus and 2 mitoses/10hpf (x400 magnification) were observed.

Histological diagnosis
Presumptive Hepatocellular carcinoma, clear-cell variant.

Special stains:
Periodic acid Schiff (PAS) stain with and without diastase digestion and Masson's trichrome (MT) stain were both performed on formalin fixed, paraffin embedded tissue. Oil-red O stain was performed on formalin fixed tissue embedded in OCT and sectioned on a cryostat. PAS showed abundant glycogen; MT showed no significant fibrosis. Oil-red O stain confirmed abundant intracellular lipid.

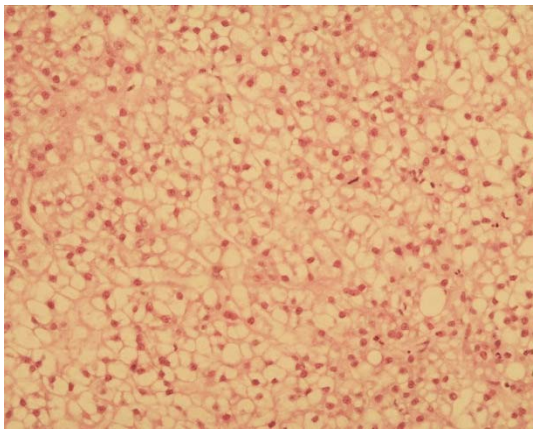

Figure 5: Sheets of cells with vacuolated cytoplasm and lack of hepatic architecture. One mitotic figure is visible in this field. (200x Magnification, Haematoxylin and Eosin).

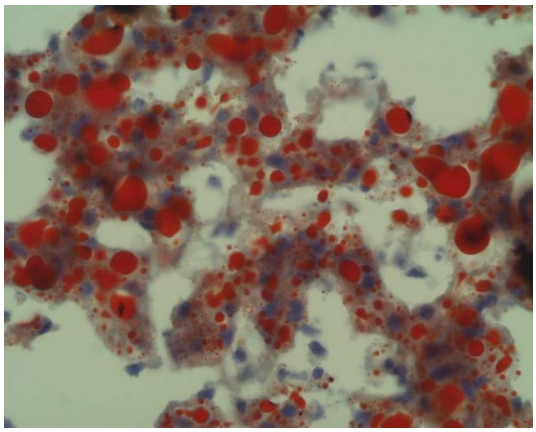

Figure 6: Oil-red O stain demonstrates abundant lipid (red) (400x Magnification, Oil-red O)

Immunohistochemistry:
Hep Par 1 (Hepatocyte antigen) staining labeled the cytoplasm diffusely positive, confirming the hepatocellular nature of the cells.

Patient Outcome
Laparotomy was performed to attempt complete excision of the mass, but due to its large size and proximity to major vessels and structures it was deemed non-resectable. Multiple further nodules were also present throughout the entire liver. A biopsy taken from one of the nodules in the left lateral liver lobe revealed a focus of nodular hyperplasia. The liver surrounding the hyperplastic nodule showed marked multifocal to coalescing random hepatocellular vacuolar degeneration, with mild periportal fibrosis identified on H & E. MT stain on this section showed moderate periportal and occasionally bridging fibrosis.
The patient was discharged with palliative care only, and was subsequently euthanised eight months after presentation. Post-mortem was not performed.

DISCUSSION

The finding of a large (approximately 8x11cm) single hepatic mass identified on ultrasound and CT raised suspicion for neoplasia. Although the size of an ultrasonographically detected focal lesion of the liver is positively correlated with malignancy[1], there is some overlap in size and echogenicity between neoplastic and non-neoplastic lesions and neoplasia is therefore not thought to be reliably predicted or ruled out based on liver ultrasound alone[2].

Non-neoplastic focal hyperplastic lesions in canine livers include nodular hyperplasia or regenerative nodules[3]. Although non-neoplastic focal lesions often measure less than 3cm in diameter, they can occasionally be larger[2,3]. Nodular hyperplasia is common in older dogs, can arise in otherwise healthy livers, and is often present as multiple, randomly distributed, nodular proliferations[4]. In contrast, regenerative nodules represent hyperplasia of surviving hepatocytes against a background of hepatic injury, atrophy and fibrosis[3].

Hepatic neoplasms may arise from within the hepatic parenchyma or be metastatic. The pattern of distribution of primary hepatic tumours is frequently described as massive (single large mass affecting one lobe, possibly extending into adjacent lobes), nodular (in some clinical texts this is indicated to describe multifocal nodules affecting several lobes) or diffuse[5,6,7].

On FNA, the cells were identifiable as hepatocytes, without any other significant populations of cells present, so given the size of the lesion hepatocellular neoplasia (adenoma or hepatocellular carcinoma) was presumed. If cellular atypia is mild or absent, differentiation between well differentiated hepatocellular carcinoma (WD HCC), adenomas, nodular hyperplasia and normal liver is challenging on cytology, as architecture is inherently not evaluated. One recent study described cytologic features of WD HCC and compared these to those of non-neoplastic, non-nodular liver. In that study, multinucleation and presence of multiple nucleoli, both of which were found in the presented case, were reported in a proportion of the WD HCC, while neither were features of the samples from non-neoplastic non-nodular liver.[8] That study did not however compare cytological features of adenoma or nodular hyperplasia.

A cytological finding which was present on all and particularly prominent on one of the smears, was marked, frequently macrovesicular, vacuolation of the hepatocytes, with abundant lipid vacuolation in the background. As confirmed by Oil-red O stain, the hepatocytes in the present case contained abundant lipid. As hepatocytes play a key role in lipid metabolism, lipid accumulation can occur secondary to a variety of insults, including hypoxia, toxins and endocrine disease[4]. Lesser amounts of glycogen were also present, as confirmed by PAS stain with diastase digestion.

The histopathological diagnosis of a HCC was based on the arrangement of the hepatocytes in sheets, lacking the typical 1-2 cell wide trabecular arrangement of the liver, lack of portal tracts and the presence of mitotic figures.

Histologically, HCC can range from well differentiated (WD) to poorly differentiated[5]. While poorly differentiated HCC can show marked cellular pleomorphism, cells in WD HCC may resemble normal hepatocytes, which can complicate the distinction from adenomas[4]. Diagnostic criteria for differentiation of WD HCC from adenomas and nodular hyperplasia appear not to be well defined[5]. Generally, hyperplastic nodules retain all the elements of normal lobular architecture, showing only greater separation of the portal triads and central veins by cords of hyperplastic hepatocytes 1-2 cells in thickness, while adenomas only

contain very scarce portal tracts and central veins[4], possibly elements of residual architecture, and show more marked disarray of the hepatocyte cords[9]. In WD HCC, clear criteria of malignancy include invasion at the margin of the adjacent compressed tissue[4] and invasion of portal vessels[3]. Due to limitations of the sampling technique (tru-cut), the margins of this lesion were not available for assessment to confirm invasion.

A prominent feature, both cytologically and histologically, was the macrovesicular vacuolar change of hepatocytes. Histologically it is not uncommon to see focal vacuolar change within HCC, consisting of either lipid, glycogen, or both. A clear cell variant, in which the majority of the hepatocytes show marked vacuolation, is however less common[5,10]. A study by Patnaik et al. who describe this variant, classifies 5 out of 57 cases of HCC (approximately 9%) as clear cell subtype[10]. The exact prevalence in dogs may however be difficult to establish, as this subclassification appears to be inconsistently applied by histopathologists and criteria for diagnosis (such as the percentage of area affected by vacuolar change) may vary.
The marked macrovesicular vacuolation on multiple tru-cut biopsies prompted a diagnosis of a clear cell variant of HCC in the presented case. Excisional biopsy would however be required to reliably assess the percentage of the tumour affected by the lipid change.

To our knowledge, the cytologic appearance of a clear cell variant of HCC has not been described in the dog. Lipid accumulation in hepatocytes, lipidosis or in humans steatosis, may be focal or diffuse and can occur in a variety of conditions such as hypoxia (e.g. secondary to anaemia or venous congestion), nodular hyperplasia, diabetes mellitus, vitamin E deficient diets[3] and in response to aflatoxicosis[11] or other toxins. Idiopathic hyperlipoproteinaemia can also cause hepatic lipidosis in Miniature Schnauzer dogs[3]. As hepatocellular carcinomas, including those of clear cell variant, may contain significant amounts of lipid[3,5], these should be a further differential to be considered for lipid-rich liver aspirates.

In humans, the variant known as clear-cell hepatocellular carcinomas is rare, yet well-described cytologically[12,13], and requires differentiation from other clear-cell carcinoma types including renal clear cell carcinoma metastatic to the liver[12,13]. Although to the authors' knowledge clear cell renal carcinoma is not described in the dog and the cells were morphologically consistent with hepatocytes, immunohistochemistry for Hep Par 1 (Hepatocyte antigen) was performed on this mass to confirm hepatocellular origin of the cells. Hep Par 1 has been shown to be a useful marker to confirm hepatocellular origin of canine hepatic neoplasms[14].
The possible clinical benefit of differentiating a clear cell variant from other HCC is entirely unknown in dogs, whereas controversially[15] in humans, some studies have suggested this subtype may carry a different prognosis to other HCC[16] . HCC in humans typically occurs in individuals with chronic liver disease and risk factors include alcohol use, Hepatitis B and C virus infections with or without cirrhosis[17]. The extent of the underlying disease as well as the amenity of the neoplasm to resection affect treatment options and therefore prognosis[17]. The presented case appeared to have diffuse hepatic disease (patchy hepatocellular vacuolar degeneration and fibrosis), for which a specific aetiology was not identified, and it is unclear whether or not this may have contributed to the development of a HCC.

KEY POINTS:

- HCC is a differential in any focal or more extensive lesion identified in the liver, and cytologists should be aware of the possibility of this neoplasm in association with macrovesicular change in hepatocytes and free lipid in the background of hepatic aspirates.
- Interpretation of mass lesions in the liver often requires information on clinical presentation, imaging findings, and architectural assessment by histopathology. In the case of hepatocellular neoplasia it remains essential for all but the most poorly differentiated neoplasms.

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