Received: 12 September 2022 | Revised: 26 October 2022 | Accepted: 8 November 2022

DOI: 10.1111/vcp.13221

Veterinary Clinical Pathology
An International Journal of Laboratory Medicine
WILEY

CASE REPORT

Needle tract seeding and malignant transformation of hepatocellular adenoma into well-differentiated hepatocellular carcinoma in a dog

Oriol Jornet-Rius[1] | Beatriz Agulla[1] | María Cristina López[2] | Claudia Viñeta[2] | Alicia García-Ferrer[2] | Bárbara Serrano[3] | Alberto Marco[3] | Anna Palomares[2] | Rosa Novellas[1,2] | Yvonne Espada[1,2] | Xavier Roura[2] | Laia Solano-Gallego[1]

[1]Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona, Spain

[2]Hospital Clínic Veterinari, Universitat Autònoma de Barcelona, Barcelona, Spain

[3]Servei de Diagnòstic de Patologia Veterinària, Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona, Spain

Correspondence
Laia Solano-Gallego, Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
Email: laia.solano@uab.cat

Abstract

An 11-year-old neutered female Golden Retriever was referred for investigation of marked increases in liver enzyme activities. Abdominal ultrasound revealed a large pedunculated liver mass. Diagnosis of hepatocellular adenoma (HCA) was made when the mass was excised after a first unsuccessful attempt through ultrasound-guided core-needle biopsy. One and a half years after presentation, a nodule embedded between muscles of the abdominal wall appeared. The mass was first diagnosed as a well-differentiated hepatocellular carcinoma (HCC) through cytologic examination, which was later confirmed with histopathology. Ki 67 immunostaining of the abdominal wall nodule showed an increased immunoreactivity compared with the liver mass. Therefore, the present case documents the first needle-tract seeding of a hepatocellular epithelial tumor with possible malignant transformation of HCA into a well-differentiated HCC in a dog.

KEYWORDS
canine, implantation, liver, metastasis, neoplasia

1 | CASE PRESENTATION

An 11-year-old neutered female golden retriever was referred with a history of a marked increase in liver enzyme activities. At presentation, complete blood count (CBC) and biochemistry panel showed a mild microcytic normochromic nonregenerative anemia (hematocrit 33.7%, reference interval [RI] 37.3%–61.7%; mean corpuscular volume 54.6 fL, RI 61.6–73.5 fL) and elevation of alanine aminotransferase (599 U/L, RI 10–94 U/L), gamma-glutamyl transferase (51 U/L, RI 10–15 U/L) and alkaline phosphatase (3725 U/L, RI 10–150 U/L). The abdominal ultrasound revealed a heterogeneous mass in the mid-abdomen. The mass measured approximately 10.5 × 6.7 cm and was pedunculated and continuous with the caudal margin of the left liver lobe.

An ultrasound-guided biopsy of the mass was performed using a semiautomatic 14-gauge core needle (ARGON Medical Devices, Athens, TX, USA). A small skin incision was performed slightly right of the midline to facilitate needle passage, and once the needle was positioned in the lesion, the trigger was released, and the needle was removed. The core specimen was teased from the needle and placed in formalin for histologic assessment. This procedure was performed twice at the same incision site. A small quantity of free fluid was observed after the core biopsy. The patient was monitored, and additional free fluid was not detected on repeated ultrasound

examinations. The histologic examination showed a diffuse mild inflammatory process mainly composed of macrophages and scarce neutrophils, hepatic trabeculae were regular in thickness, and hepatocytes were morphologically normal. The absence of portal triads and the small sample size made it impossible to establish a definitive diagnosis. Treatment with a hepatic protector was started (Samilyn medium size; two tablets once per day).

The dog was reevaluated 3 months after the initial presentation. The owners described episodes of tachypnea interpreted as pain. The physical examination was normal. CBC, biochemistry, thoracic radiographs, and abdominal ultrasound were performed. A persistent marked increase in liver enzyme activities was detected in the biochemistry profile, and the ultrasound showed a mild increase in the size of the liver mass (12 × 8.6 cm), with a similar appearance. Given these changes, an exploratory laparotomy with a partial hepatectomy was performed. Before the mass excision, a surgical stapling of the liver parenchyma and vessels was performed. No adhesions were observed between the liver mass and other abdominal structures. Eventually, the liver mass was sent for histopathologic evaluation.

The histopathologic examination of the mass showed a nonencapsulated, moderately cellular, neoplastic epithelial proliferation that was clearly demarcated and well differentiated from the adjacent normal liver (Figure 1A). Neoplastic cells formed regular trabeculae of 2–3 cells thick and were separated by sinusoids (Figure 1B). Neoplastic cells were well differentiated and had focal areas of finely vacuolated cytoplasm and round nuclei with single nucleoli. Mild anisocytosis and anisokaryosis were seen, with 0–1 mitotic figures/HPF and mild positivity for Ki 67, a marker of cellular proliferation (Figure 1E). A diagnosis of hepatocellular adenoma (HCA) was made.

Seventeen months after the core-needle biopsy, the presence of an adhered 3 cm subcutaneous nodule was noted at the previous core-needle biopsy site. An abdominal ultrasound was performed and found to be unremarkable. Ultrasound of the nodule showed that it was embedded into the muscular layers of the ventral abdominal wall and measured 1.96 × 2.16 cm (Figure 2A). An ultrasound-guided fine-needle aspiration (FNA) of the nodule using a 23-gauge needle was performed and sent for cytopathologic examination. On cytology, many sheets of polygonal to round hepatocytes with mild to moderate atypia were seen, including moderate anisokaryosis, frequent binucleated and multinucleated cells, macrokaryosis, multiple nucleoli, and atypical mitotic figures. These findings were suggestive of a proliferation of hepatocytes with mild to moderate atypia and a cytologic diagnosis of well-differentiated hepatocellular carcinoma (WD-HCC; Figure 3A–F).

CBC and biochemistry results were within normal limits, with no abnormalities found on thoracic radiographs. On full-body computed tomography, a soft tissue-attenuating nodule of 2 cm in diameter was identified in the rectus abdominis muscle, affecting both internal and external layers without extending into the peritoneal cavity (Figure 2B,C). The lesion showed a moderate heterogeneous contrast enhancement and was located at the level of the third lumbar vertebra.

Options were discussed with the owners, and an en-bloc surgical resection of the muscular nodule was made using an elliptical incision with 2 cm macroscopic lateral and deep margins around the lesion. On histopathology, the mass consisted of a partially encapsulated, moderately cellular, lobulated neoplastic growth composed of irregular thick trabeculae associated with a moderate amount of fibrous desmoplasia (Figure 1C). Multifocal, randomly distributed areas of coagulative necrosis and hemorrhage were observed (Figure 1D). Neoplastic cells were well differentiated, and mild to moderate anisocytosis and anisokaryosis were observed. There was a low mitotic index (2–3 mitotic figures/HPF) with occasionally aberrant mitotic figures. Ki 67 immunostaining showed a highly increased cellular activity (Figure 1F). A diagnosis of well-differentiated trabecular hepatocellular carcinoma (HCC) was made.

The dog recovered without any complications, and 8 months after surgery, the dog showed no clinical signs, and the physical examination was unremarkable. Unfortunately, a complete staging, including imaging diagnostics, was not performed.

2 | DISCUSSION

Hepatic neoplasia is an infrequent condition in dogs accounting for between 0.6% and 1.3% of all canine neoplasms.[1,2] HCC is the most frequent primary hepatic neoplasm in dogs; however, HCA is an unusual condition. Both neoplasms usually appear in dogs older than 10 years, and sex or breed predispositions do not seem to be present.[1,2] Dogs with hepatic neoplasia can show nonspecific clinical signs.[1,2] However, like in the present case, up to 50% of dogs with these neoplasms show no clinical signs and are diagnosed during the investigation of increased liver enzyme activities.[3] Although the physical examination is often unremarkable, a cranial abdominal mass can be detected in up to 75% of dogs with a liver tumor.[2] Clinicopathologic findings are also nonspecific; therefore, they cannot distinguish between neoplastic and nonneoplastic liver disease. Nonregenerative anemia, leukocytosis, and increased liver enzyme activities are the most common laboratory findings.[1,2]

Differential diagnoses of hepatic masses in dogs include nodular hyperplasia, regenerative nodules, HCA, HCC, bile duct carcinoma, hepatic carcinoids, abscesses, and metastatic neoplasia. Therefore, in veterinary medicine, morphologic evaluations of the liver remain necessary to establish a definitive diagnosis.[4] In this case, a first diagnostic attempt was carried out through an ultrasound-guided core-needle biopsy. The regularity of the trabeculae and the morphologic features of the hepatocytes resembled normal liver parenchyma, but the absence of portal spaces made it impossible to establish whether it was a well-differentiated benign neoplastic growth (adenoma) or hyperplastic nodule. However, in human medicine, a diagnosis is primarily based on computed tomography and magnetic resonance imaging findings, and biopsy is only used when the imaging pattern is not fully concordant with the diagnostic criteria.[5] A study in dogs comparing different methods of liver core-needle biopsies using

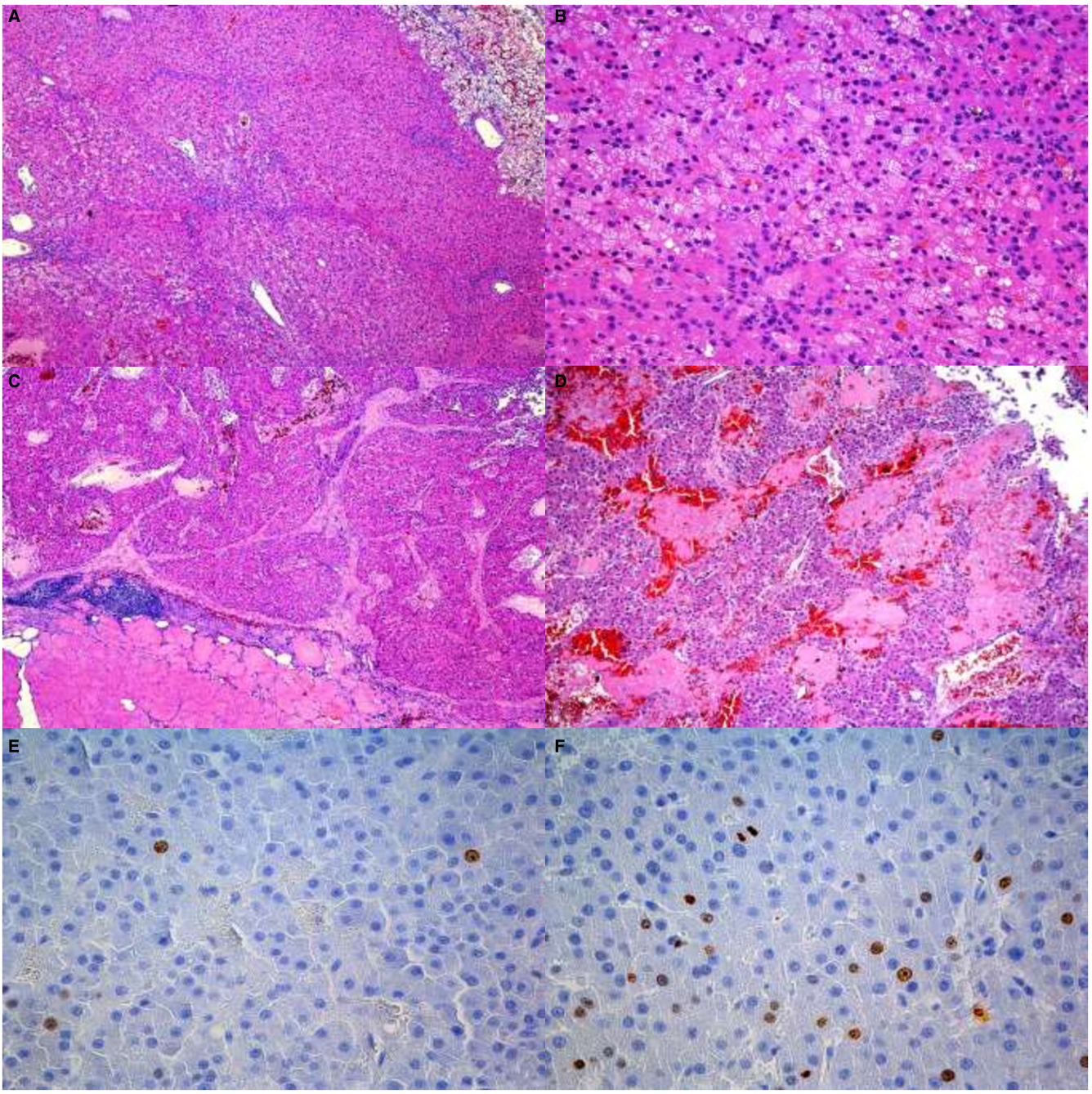

FIGURE 1 Histopathologic examination of the hepatic mass and intramuscular abdominal wall nodule in a dog with hepatocellular carcinoma. (A, B) Hepatocellular adenoma. The neoplasm is well demarcated with adjacent normal liver and formed pseudolobular structures composed of regular thick trabecula. (C) Hepatocellular carcinoma effacing muscle and adipose tissue and showing loss of lobar architecture, irregular thickness of trabeculae (>5 hepatocytes thick), and moderate fibrous desmoplasia. (D) Randomly distributed areas of lytic necrosis and hemorrhage within the intramuscular hepatocellular carcinoma. (E) Ki-67 immunostaining of the hepatic mass showed mild positivity (on average 2.9 positive nuclei/HPF). (F) Ki-67 immunostaining of the intramuscular mass showed a highly increased positivity on average 21.1 positive nuclei/HPF.

different needle sizes with paired necropsy biopsies found a low agreement between core-needle and wedge biopsies.[6] This observation was related to the small size of the samples obtained with core-needle biopsies. Therefore, obtaining 3–12 portal triads and multiple biopsies is recommended to enhance the likelihood of a correct diagnosis.[6]

In the present case, the histologic features of the surgically excised liver mass were consistent with HCA. However, one and a half years after the core-needle biopsy, a slowly growing abdominal wall nodule appeared in the region of the needle tract. This mass was diagnosed as a well-differentiated HCC cytologically, and subsequently, the final diagnosis was confirmed by histopathologic

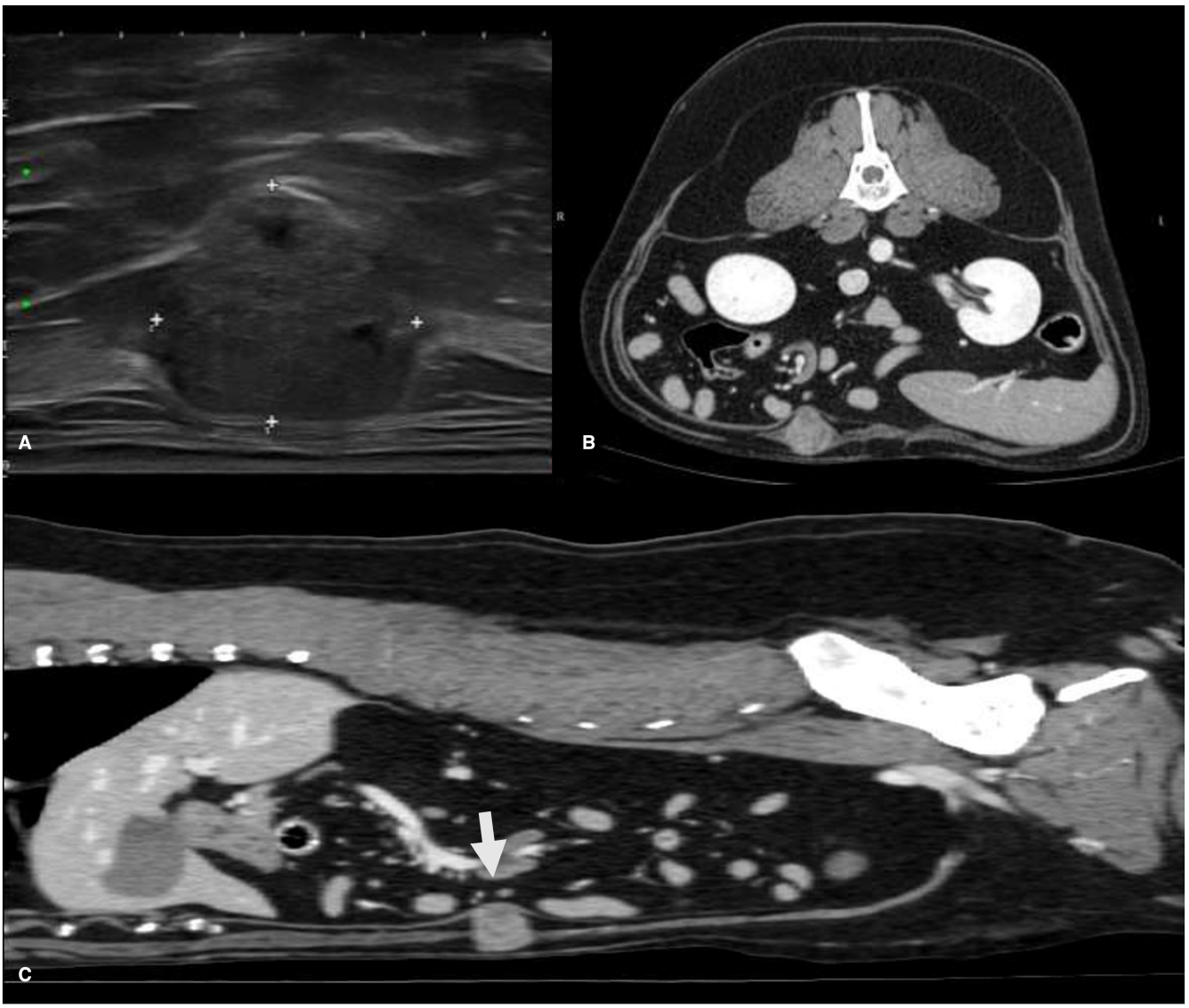

FIGURE 2 CT and ultrasound images of the abdomen of a dog with hepatocellular carcinoma 17 months after the core-needle biopsy. (A) Ultrasound with a 15 MHz linear transducer. The nodule was mildly heterogeneous, hypoechoic, well defined, and embedded in the rectus abdominis muscle. (B) A postcontrast transverse image in a soft-tissue window at the level of the third lumbar vertebra. The nodule was located in the rectus abdominis muscle and did not extend into the peritoneal cavity. No further nodules were identified. (C) Postcontrast sagittal CT reconstruction in the soft tissue window. The soft tissue-attenuating nodule (white arrow) is located around 8 cm caudal to the liver. CT, computed tomography.

evaluation. Additionally, both specimens were later reviewed by another ECVP-boarded pathologist in a blinded fashion, confirming both diagnoses.

Malignant transformation of an HCA into an HCC is a rare but well-known complication in human medicine[7]; however, to the authors' knowledge, this is the first report of possible malignant transformation associated with needle tract seeding in dogs. Malignant transformation has been reported in 4.2% of human patients with HCA.[7] In human medicine, although the natural history of this progression is not well defined, multiple risk factors have been associated with malignant transformation, including male gender, a tumor size >5 cm, oral contraceptive intake, and genetic mutations.[7,8] In this dog, the presence of a 10-fold increase in Ki 67 expression by the abdominal wall mass compared with the initial liver mass could support the hypothesis of a malignant transformation during the seeding process. However, the ability of the Ki 67 to differentiate HCA from HCC has only been evaluated in human liver samples.[9] Although most HCA tumors have had low Ki 67 expression (<4%), up to 50% of HCC cases had Ki 67 expression <4%, limiting its usefulness.[9] A study in dogs evaluating the usefulness of Ki 67 in the cytologic identification of liver tumors found a significant increase in Ki 67 expression in hepatic neoplasia compared with nontumoral lesions, suggesting that the concurrent use of cytology and Ki 67 immunocytochemistry could improve the diagnostic accuracy of identifying liver neoplasia.[10] Nevertheless, this study did not include any HCA cases; therefore, the ability of using Ki 67 expression to

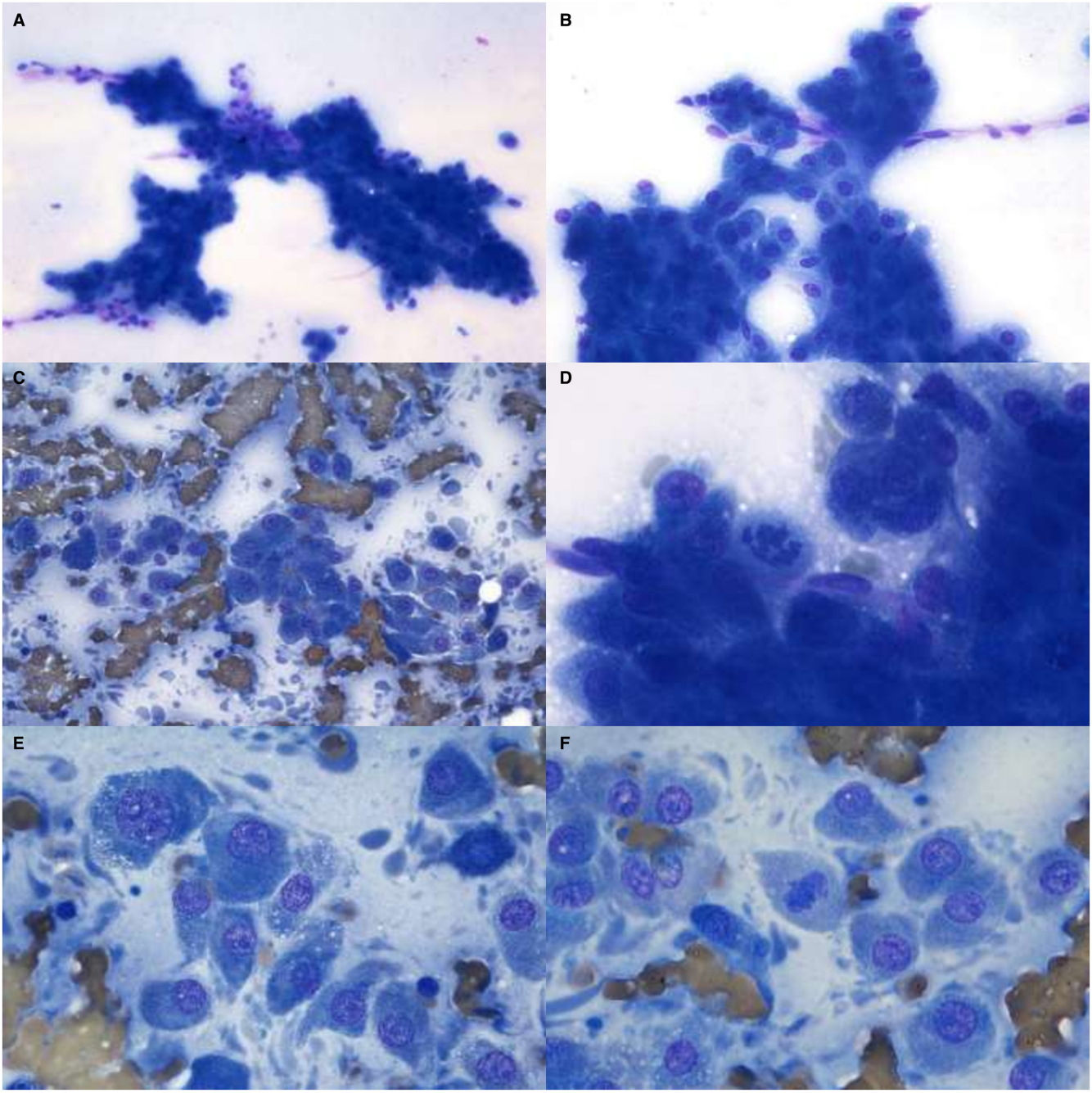

FIGURE 3 Well-differentiated hepatocellular carcinoma in the abdominal wall of a dog. (A, B, D) Fine-needle aspiration of the abdominal wall mass. (C, E, F) Imprint touch cytology of the same mass. Modified rapid Romanowsky-stain (Quick Panoptic). (A, B) Sheets of polygonal to rounded epithelial cells with a perivascular arrangement. (C, D) Moderate to high nucleus-to-cytoplasm ratio and mild to moderate anisocytosis and anisokaryosis. (D–F) Round centrally placed nuclei with coarsely stippled chromatin. Amphophilic with distinct cytoplasmic borders. Occasional clear intracytoplasmic vacuoles are present. (D) A Hepatocellular aggregate shows a perivascular arrangement and an atypical mitotic figure (center). (E) The group of hepatocytes shows moderate anisocytosis and anisokaryosis, with one to multiple prominent nucleoli. A macronucleus is observed (top right). (F) An atypical mitotic figure is seen at the center of the image.

differentiate between HCC and HCA remains unknown in veterinary medicine.[10]

The morphologic features of a WD-HCC often overlap with those of benign HCA neoplasms, and less frequently, benign proliferations such as nodular hyperplasia, and regenerative nodules, are challenging to distinguish on either histopathologic or cytologic examinations. Histologic evidence of extrahepatic metastasis and vascular/adjacent parenchyma invasion are key diagnostic criteria for malignancy.[2] However, these features are not always present and; therefore, the presence of other features such as cellular pleomorphism, mitotic figures, multiple nucleoli, and variation in thickness of the trabeculae, has to be evaluated.[11] Useful cytologic features in

the diagnosis of WD-HCC are the dissociation of hepatocytes, acinar or palisading arrangements, the presence of naked nuclei and capillaries, multinuclearity, increased N:C ratios, and mild anisocytosis and anisokaryosis.[12] Therefore, we cannot completely rule out that the initial diagnosis of HCA, in this case, might have been a misdiagnosis of a well-differentiated HCC with subsequent seeding.

Needle-tract seeding is a known complication associated with percutaneous needle biopsies in both humans and animals, and although its incidence is rare, the development of needle tract metastases following biopsies has been described in almost every type of neoplasm in human medicine.[13] In veterinary medicine, it has been described in the urinary bladder, urethral, and prostatic transitional cell carcinoma[14–17] pancreatic carcinoma,[18] pericardial mesothelioma,[19] renal cell carcinoma,[20] carcinomatosis,[21] and pulmonary adenocarcinoma.[16,22] Seeding of HCC along the needle tract is widely described in human medicine, and although the incidence of needle-tract seeding was initially thought to be high (up to 5% of cases, depending on the study),[23] a recent larger study described a low incidence (0.25%) of needle track seeding in patients with HCC.[24] The time needed for the development of seeded tumors is variable and does not seem to affect survival.[23] Several factors are associated with a higher risk of implantation along the needle tract, including multiple needle passes, use of larger-gauge needles with a greater cutting area, the degree of histologic differentiation (higher risk in patients with WD-HCC due to a longer survival), and thickness of the liver parenchyma along the needle tract.[23] The small size of the tumor has also been related to an increased risk of implantation, but this is explained since, in human medicine, imaging findings of small HCC are nonspecific, and a greater number of small HCC tumors are biopsied than larger ones.[25] In the present dog, the biopsy was performed with a 14-gauge needle and a single pass; however, the large tumor size, superficial position, and the well-differentiated nature could be the reasons why the implanted tumor was detectable.

Finally, although veterinarians should be aware of the risk of needle tract implantation after percutaneous biopsies, the frequency seems to be generally low, and the benefits outweigh the cons compared with the valuable information obtained.[23] Due to the low agreement between core-needle and wedge biopsies, multiple core-needle samples should be taken. Moreover, ultrasound-guided FNAs should always be pursued as a first step when dealing with hepatic masses and nodules, prior to more invasive liver biopsy techniques.

In conclusion, this case report documents the first needle-tract seeding of a hepatocellular epithelial tumor and the possible malignant transformation of HCA into HCC in a dog.

DISCLOSURE

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

ORCID

Oriol Jornet-Rius https://orcid.org/0000-0001-7495-3961
Beatriz Agulla https://orcid.org/0000-0003-0136-8264
María Cristina López https://orcid.org/0000-0003-2744-4077
Claudia Viñeta https://orcid.org/0000-0001-8668-5538
Alicia García-Ferrer https://orcid.org/0000-0003-1238-522X
Bárbara Serrano https://orcid.org/0000-0003-4062-6211
Alberto Marco https://orcid.org/0000-0003-1680-0822
Anna Palomares https://orcid.org/0000-0002-8334-7346
Rosa Novellas https://orcid.org/0000-0003-2392-9202
Yvonne Espada https://orcid.org/0000-0003-1556-6587
Xavier Roura https://orcid.org/0000-0002-1098-2381
Laia Solano-Gallego https://orcid.org/0000-0001-8479-4896

REFERENCES

1. Patnaik AK, Hurvitz AI, Lieberman PH. Canine hepatic neoplasms: a clinicopathologic study. *Vet Pathol*. 1980;17:553-564.
2. Trigo FJ, Thompson H, Breeze RG, Nash AS. The pathology of liver tumours in the dog. *J Comp Pathol*. 1982;92(1):21-39.
3. Balkman C. Hepatobiliary neoplasia in dogs and cats. *Vet Clin North Am: Small Anim*. 2009;39(3):617-625.
4. Warren-Smith CMR, Andrew S, Mantis P, Lamb CR. Lack of associations between ultrasonographic appearance of parenchymal lesions of the canine liver and histological diagnosis. *J Small Anim Pract*. 2012;53(3):168-173.
5. Ayuso C, Rimola J, Vilana R, et al. Diagnosis and staging of hepatocellular carcinoma (HCC): current guidelines. *Eur J Radiol*. 2018;101:72-81.
6. Kemp SD, Zimmerman KL, Panciera DL, Monroe WE, Leib MS, Lanz OI. A comparison of liver sampling techniques in dogs. *J Vet Intern Med*. 2015;29(1):51-57.
7. Stoot JHMB, Coelen RJS, de Jong MC, Dejong CHC. Malignant transformation of hepatocellular adenomas into hepatocellular carcinomas: a systematic review including more than 1600 adenoma cases. *HPB*. 2010;12(8):509-522.
8. Nault JC, Couchy G, Balabaud C, et al. Molecular classification of hepatocellular adenoma associates with risk factors, bleeding, and malignant transformation. *Gastroenterology*. 2017;152(4):880-894.e6.
9. Jones A, Kroneman TN, Blahnik AJ, et al. Ki-67 "hot spot" digital analysis is useful in the distinction of hepatic adenomas and well-differentiated hepatocellular carcinomas. *Virchows Arch*. 2021;478(2):201-207.
10. Neumann S, Kaup FJ. Usefulness of Ki-67 proliferation marker in the cytologic identification of liver tumors in dogs. *Vet Clin Pathol*. 2005;34(2):132-136.
11. Meuten DJ. *Tumors in Domestic Animals*. 5th ed. Wiley Blackwell; 2016.
12. Masserdotti C, Drigo M, Veterinario L, Marco S. Retrospective study of cytologic features of well-differentiated hepatocellular carcinoma in dogs. *Vet Clin Pathol*. 2012;41(3):382-390.
13. Klopfleisch R, Sperling C, Kershaw O, Gruber AD. Does the taking of biopsies affect the metastatic potential of tumours? A systematic review of reports on veterinary and human cases and animal models. *Vet J*. 2011;190(2):e31-e42.
14. Reed LT, Knapp DW, Miller MA. Cutaneous metastasis of transitional cell carcinoma in 12 dogs. *Vet Pathol*. 2013;50(4):676-681.
15. Higuchi T, Burcham GN, Childress MO, et al. Characterization and treatment of transitional cell carcinoma of the abdominal wall in dogs: 24 cases (1985–2010). *J Am Vet Med Assoc*. 2013;242(4):499-506.
16. Vignoli M, Rossi F, Chierici C, et al. Needle tract implantation after fine needle aspiration biopsy (FNAB) of transitional cell carcinoma of the urinary bladder and adenocarcinoma of the lung. *Band*. 2007;149:314-318.
17. Nyland TG, Wallack ST, Wisner ER. Needle-tract implantation following us-guided fine-needle aspiration biopsy of transitional

cell carcinoma of the bladder, urethra, and prostate. *Vet Radiol Ultrasound*. 2002;43(1):50-53.
18. Jegatheeson S, Dandrieux JRS, Cannon CM. Suspected pancreatic carcinoma needle tract seeding in a cat. *J Feline Med Surg Open Rep*. 2020;6(1):205511692091816.
19. Morgan KRS, Dominic CG, Beeler-Marfisi J, et al. Presumptive seeding metastasis of pericardial mesothelioma following repeated pericardiocentesis in a dog. *Can Vet J*. 2019;60(9):972-975.
20. Livet V, Sonet J, Saadeh D, Pillard P, Carozzo C. Needle-tract implantation after fine-needle aspiration biopsy of a renal cell carcinoma in a dog. *Vet Rec Case Rep*. 2017;4(2):e000349.
21. Moore AR, Coffey E, Leavell SE, et al. Canine bicavitary carcinomatosis with transient needle tract metastasis diagnosed by multiplex immunocytochemistry. *Vet Clin Pathol*. 2016;45(3):495-500.
22. Warren-Smith CMR, Roe K, de La Puerta B, Smith K, Lamb CR. Pulmonary adenocarcinoma seeding along a fine needle aspiration tract in a dog. *Vet Rec*. 2011;169(7):181.
23. Silva MA, Hegab B, Hyde C, Guo B, Buckels JAC, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut*. 2008;57(11):1592-1596.
24. Szpakowski JL, Drasin TE, Lyon LL. Rate of seeding with biopsies and ablations of hepatocellular carcinoma: a retrospective cohort study. *Hepatol Commun*. 2017;1(9):841-851.
25. Kim SH, Lim HK, Lee WJ, Cho JM, Jang HJ. Needle-tract implantation in hepatocellular carcinoma: frequency and CT findings after biopsy with a 19.5-gauge automated biopsy gun. *Abdom Imaging*. 2000;25(3):246-250.

How to cite this article: Jornet-Rius O, Agulla B, López MC, et al. Needle tract seeding and malignant transformation of hepatocellular adenoma into well-differentiated hepatocellular carcinoma in a dog. *Vet Clin Pathol*. 2023;52:507-513. doi:10.1111/vcp.13221