

ORIGINAL ARTICLE

Size of canine hepatocellular carcinoma as an adverse prognostic factor for surgery

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ABSTRACT

Objective: Liver neoplasms are problematic among small domestic animals. The etiological cause of hepatocellular carcinomas in domestic animals is still unknown although it is believed that chronic infections and toxic substances can affect the development of this type of tumor. This study aimed to analyze the clinical and morphological characteristics of canine hepatocellular carcinoma.

Materials and methods: In total, 6,958 cancer operations were performed in the clinic. Liver tumors were detected in 123 dogs *in vivo* and 375 dogs postmortem. All animals with suspected liver neoplasm were assessed, including history, clinical examination, complete blood count, biochemical blood tests, radiographic examination, and ultrasound with a biopsy for performing cytological and histological analyses.

Results: Hepatocellular carcinomas have nonspecific clinical manifestations, also a characteristic aspect of other tumors of the hepatobiliary system. The hematological changes have an impact on the prognosis, and biochemical abnormalities reflect the changes in liver activity. The cytological diagnosis of hepatocellular tumors is difficult because of hepatocyte atypia in highly differentiated carcinomas. Finally, a histological examination was performed in all the dogs diagnosed with hepatocellular carcinoma.

Conclusion: Hematological changes in dogs with hepatocellular carcinoma affect their prognosis. Biochemical abnormalities of this pathology reflect the changes in liver activity, not indicating a specific pathology. However, an increase in the activity of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase is an unfavorable prognostic sign. In this study, five of seven dogs with a tumor size of more than 5.0 cm had a life expectancy of 30, 51, and 91 days, suggesting that the size of the tumor is an adverse prognostic factor.

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Introduction

Tumors of the hepatobiliary system in dogs make up 1% of all neoplasms [1,2]. It is believed that dogs have a higher incidence of hepatocellular cancer than other animal species [3–5]. Hepatocellular carcinomas are the most common liver neoplasms in dogs, making up 35%–60% of all canine primary liver tumors [6–8]. The prognosis for hepatocellular carcinoma depends on surgical outcomes, the size of the liver part involved, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels, as well as the AST-to-ALT ratio [4]. With one lobe damage and complete resection, the prognosis is considered favorable and is

estimated at > 1,460 days. In contrast, increasing liver enzyme activity is an unfavorable prognosis factor [9].

Liver tumors do not usually have unique clinical manifestations. Therefore, laboratory diagnostic methods (e.g., morphological methods for analyzing cell composition) are particularly relevant for neoplastic and nonneoplastic lesion differentiation [10]. Although liver tumors cannot normally be diagnosed by clinical signs, blood tests, or abdominal X-rays, they are easy to detect using abdominal ultrasound and computed tomography (CT). Unfortunately, modern diagnostic images (i.e., CT) are rarely used in canine species, due to their high cost and the need for sedation or anesthesia, which is undesirable

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for older dogs or those with progressive diseases. Besides, modern diagnostic images may not distinguish malignant tumor from benign ones [11]. The differentiation of metastatic tumors from primary tumors is an important prognostic factor, which indicates a decrease in lifespan in dogs with metastatic liver lesions [2,4]. This study aimed to analyze the clinical and morphological characteristics of canine hepatocellular carcinoma.

Materials and Methods

Ethical approval

This study was carried out in accordance with the State program of Peoples' Friendship University of Russia (RUDN University). A selective research on animals was conducted after receiving the approval from the Bioethics Commission SREC PFUR (Bioethics Commission Opinion No 14/03/2019).

Animals

Dogs admitted from November 2012 to August 2018 were included in this study. During this period, 6,958 cancer operations were performed in the clinic. Liver tumors were detected in 123 dogs *in vivo* and 375 dogs postmortem. Surgery was performed in 40 animals with various liver neoplasms. Hepatocellular carcinomas were morphologically detected in 18 of 40 (45%) dogs. In the majority of subjects, when liver tumors were detected, treatment was not carried out and was considered unpromising due to the metastatic process development in other organs and unsatisfactory general conditions.

The average age of subjects was 11.6 years, and the average body weight was 16.4 kg. The male:female ratio was approximately 1:1 (8 males and 10 females). The breeds were Metises (7/18), Poodles (4/18), Cocker Spaniels (3/18), Collies (2/18), German Shepherds (1/18), and Rottweilers (1/18).

Study design

All animals with suspected liver neoplasms underwent a comprehensive examination, which included history, clinical examination, palpation and percussion of the abdominal cavity, complete blood count (CBC), biochemical blood tests, radiographic examination, and ultrasound with a possible biopsy for performing cytological and histological analyses. In the presence of a liver neoplasm and surgical intervention, intraoperative ultrasound was used to detect interoperative nodes.

Radiography of the chest cavity was performed on a stationary X-ray machine (EDR 750, power of 120 kV, and current of 2 A), in case of previously performed oncological operations of any location, suspected cancer at the time

of admission, and symptoms of heart or respiratory failure. The exposure time was 0.1 s at 65–90 kV/150 mA (Fig. 1).

This study was performed on all animals studied. Ultrasounds were performed on the “DC 6-Mindray” and “Kranzburg” apparatus, equipped with multifrequency microconvex sensors with frequencies ranging from 5 to 6 MHz and 5 to 8 MHz, respectively.

The organ localization, size, and structure homogeneity were assessed. In case of heterogeneity, the volume and location of the lesion were also assessed, as well as the liver structure such as the state of parenchyma, capsules, the presence or absence of fluid under the capsule, the condition of the vessels, arteries, veins, and bile ducts (expansion, location in the parenchyma). The shape, size, wall condition, and contents of the gallbladder were also visualized. The gate area of the liver, the state of vessels, and the presence of lymph nodes were examined. In the presence of liver tumors, we also studied the localization of the lobe, size, quantity, contours, structure, and echogenicity.

The biochemical blood test was performed on the device Stat Fax 1904. The following indicators were investigated: ALT, AST, ALP, glucose, urea, creatinine, albumin, total and direct bilirubin, and gamma-glutamyltransferase.

CBC was performed using a Kohden apparatus. The number of leukocytes, platelets, erythrocytes, and hemoglobin was determined. At low platelet content, the prothrombin time and coagulogram were performed.

Spinocan needles from Braun (0.9 mm diameter and 80 mm long) were used to take a cytological sample of the pathological liver tissue when the tumor process was performed under the control of ultrasound (Fig. 2a and b).

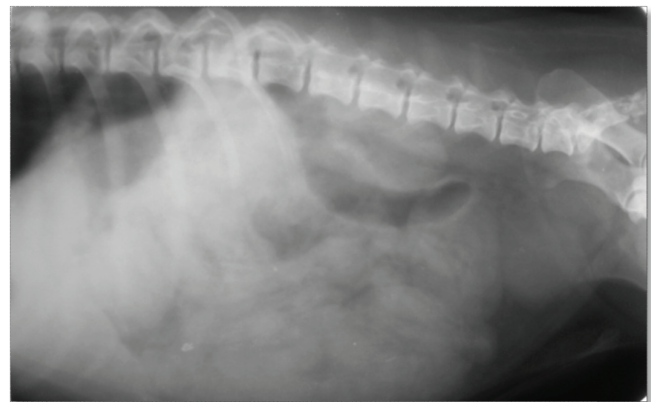


Figure 1. X-ray of the abdominal cavity in the right lateral projection: focal formation of the lobe of the liver, medium density with an uneven contour, in superposition with the stomach filled with gas and intestinal loops filled with liquid contents and a small amount of gas; without pronounced mass effect.

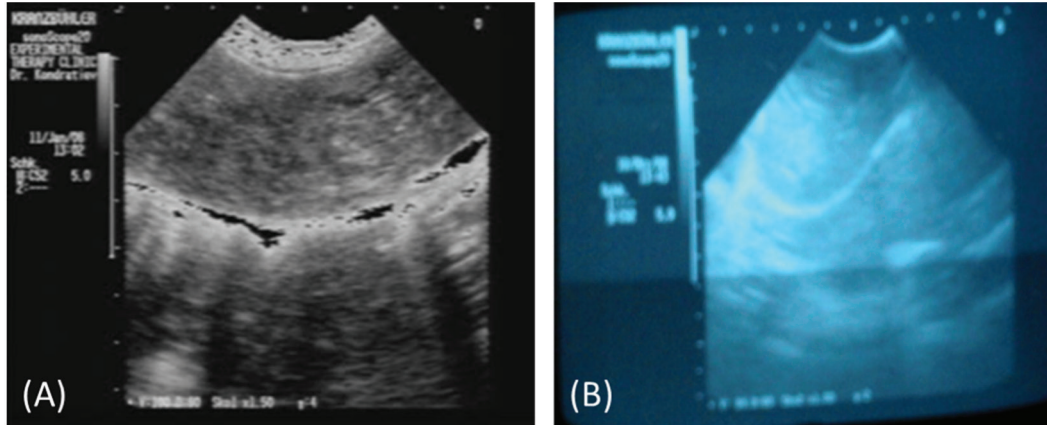


Figure 2. (A) Ultrasound examination of the liver: focal formation of a heterogeneous structure, with foci of increased and decreased echogenicity, without clear contours. (B) Intraoperative ultrasound examination of the liver: rounded formation, reduced echogenicity, heterogeneous structure, with a clear contour.

To obtain material for the histological examination, a Tru-Cut needle of size 13–16 gx was used, depending on the size of the subject. In the presence of tumor, a cytological examination was performed with a subsequent surgical intervention to obtain histological material. Tissue sampling for histological analysis was performed when the owners refused to subject their dog to surgery (e.g., if the diagnosis was unclear) and when determining the effectiveness of the therapeutic treatment.

The cytological examination was performed according to generally accepted methods. Immediately after receiving the pathological material, smears prints were prepared, fixed with May–Grunwald solution, and stained with azure-eosin according to Romanowsky–Giemsa. A microscopic examination of smears was performed using a light microscope with $\times 100$, $\times 400$, and $\times 1,000$ magnification.

An histological examination was carried out according to standard protocol, with the fixation of the material in a 10% neutral-buffered formalin solution. For histological studies, the material was obtained intraoperatively, and after euthanizing, animals were admitted to our clinic.

Data analysis

The obtained data were analyzed using variation statistics on the “Statistica” software for Windows.

Results and Discussion

Visual diagnosis plays a crucial role in determining the stage of the oncological process in canine liver tumors. Local and regional spread of metastases can be detected using ultrasound, CT-scan, magnetic resonance imaging, or laparoscopy [12,13].

An X-ray graphical examination of 9/18 subjects identified tumors in the liver projection on the X-ray picture of the abdominal cavity, made in the lateral and ventrodorsal projections. This method allows visualization of neoplasms in the presence of massive liver tumors [14,15]. Radiological findings are not specific for liver tumors and do not provide a sufficient information about the surrounding structures and tissues, usually detecting displacement of the stomach and hyperechoic masses [4,16,17].

Ultrasound examination was performed in all animals: 17/18 dogs were found to have isolated liver injury (fraction 1–2) and one dog had multiple liver tumors (> 3 lobes). In subjects with single lesions, 13 were located in the left lobes of the liver. More than a third (7/18) of neoplasms had a volume of < 5 cm, whereas 7/11 had a volume of > 5 cm. Ultrasound can provide practitioners with the tools for the visual diagnosis of hepatobiliary tumors, as it helps to visualize the lesion, determine the prevalence of the neoplasm, and evaluate the changes in neighboring organs. Unfortunately, ultrasonography cannot differentiate liver tumors or distinguish them from hyperplasia [4,12].

The biochemical blood test revealed low albumin content and an apparent increase in hepatic transaminase levels. The dynamics of biochemical and hematological blood parameters 1 day preoperatively and 30 days postoperatively are shown in Table 1. This increase in hepatic transaminase level is typical in dogs with hepatobiliary tumors. The increased liver enzyme activity is not only specific to liver tumor development but also is also associated with hepatocyte damage or the presence of biliary stasis, which can occur even in the absence of tumors [18]. In primary lesions of the liver, an increase in ALP and ALT usually occurs. In hepatocellular carcinoma, the ALT index was overestimated by a factor of 3.5 (195.7 ± 38.8) and

Table 1. Dynamics of biochemical and hematological blood parameters before and after the removal of hepatocellular carcinoma.

Value	Reference value	Before the tumor is removed	1 day after removal	30 days after removal
Biochemical value				
ALT, IU/l	8.2–57.3	195.7 ± 38.8	853.7 ± 99.8	309.1 ± 51.4
AST, IU/l	8.9–48.5	133.8 ± 43.1	372.1 ± 75.9	198 ± 56.9
Total bilirubin, umol/l	1.71–10.26	13.3 ± 7.9	4.8 ± 1.8	1.9 ± 0.3
ALP, IU/l	8–76	446.4 ± 103.9	546.1 ± 103.5	308.4 ± 81.7
Gamma-glutamyltransferase, IU/l	1.0–9.7	10.9 ± 0.9	11.9 ± 1.2	9.8 ± 1.5
Albumin, g/l	28–40	24.6 ± 2.7	22.6 ± 2.2	27.8 ± 2.6
Urea, mmol/l	3.5–9.2	15.6 ± 6.8	9.2 ± 2.3	5.6 ± 0.6
Creatinine, umol/l	44.2–141.4	92.5 ± 20.8	110.1 ± 30.9	77.4 ± 16.6
Hematological value				
Erythrocytes, 10 ¹² /l	5.65–8.87	4.8 ± 0.6	4.8 ± 0.6	6.8 ± 0.6
Hemoglobin, g/dl	13.1–20.5	10.4 ± 1.3	10.7 ± 1.4	13.4 ± 1.1
Leukocytes, 10 ⁹ /l	5.05–16.76	22.0 ± 3.6	24.9 ± 5.0	13.2 ± 0.9
Platelets, 10 ³ /l	148–484	227.5 ± 36.2	333.9 ± 51.1	305.8 ± 49.3

that of ALP by a factor of 17 (446.4 ± 103.9). The AST:ALT ratio was 0.6 on average, which may indirectly indicate the presence of hepatocellular or cholangiocellular carcinoma [19]. ALT, AST, ALP:AST ratio, and ALT:AST ratio are also prognostic factors in dogs with massive hepatocellular carcinoma [20]. A slight increase in total bilirubin (13.3 ± 7.9) and urea (15.6 ± 6.8) was also detected, as well as a slight decrease in albumin (24.6 ± 2.7). An increase in bile acids with and without an increase in ALP may indicate the presence of liver neoplasms although it does not accurately indicate a primary or metastatic lesion. Bilirubin and lactate dehydrogenase levels may also increase due to the presence of a liver neoplasm [4,21].

A hematological study revealed an increase in the number of leukocytes (22.0 ± 3.6), which may be due to the presence of secondary reactive hepatitis and tissue necrosis in the case of bulk tumor lesions in the liver [22]. A decrease in the number of red blood cells (4.8 ± 0.6) and hemoglobin levels (10.4 ± 1.3) was detected, indicating mild anemia in dogs with hepatocellular carcinoma [5,20]. The exact cause of anemia in this pathology has not only been fully elucidated but also can be associated with chronic disease or inflammation, caused by the tumor itself or due to other reasons [23–25].

After collecting anamnestic data, palpation, percussion, ultrasound, X-rays, biochemical blood tests, and CBC before surgery, a biopsy of the abnormal focus under ultrasound control was performed alongside a cytological study.

Ultrasound-controlled fine-needle aspiration biopsy is a minimally invasive method used to diagnose pathologies in various organs, including the liver [17]. However, before this procedure, it was recommended to determine the coagulation profile of the patient, as liver biopsies

caused weak-to-moderate bleeding in 5% of cases [23,24]. Furthermore, aspiration biopsy led to a correct diagnosis in 60% of the cases and in up to 90% of the cases when using a core biopsy [20,26,27].

The cytological diagnosis of hepatocellular carcinoma is difficult due to unexpressed signs of atypical hepatocytes in highly differentiated tumors. In 8/18 dogs, it was possible to diagnose hepatocellular carcinoma using cytology. Hepatocytes had a pleomorphic appearance, a high nuclear-cytoplasmic ratio, large and giant nuclei with pronounced anisokaryosis, karyomegaly, multiple nucleoli (2–3 or more) of different sizes and shapes, anisonucleosis within several nuclei, as well as the formation of acinar or palisade structures from a group of hepatocytes (Fig. 3a). In 10/18 dogs, the diagnosis was based on the alleged lack of sufficient criteria for malignancy, but this did not completely rule out the hepatocellular carcinoma. Cytologically, the samples were composed of highly differentiated hepatocytes, some had polygonal cytoplasm, some cells had vacuoles, weak-to-moderate anisocytosis was also detected, some binuclear cells were found, and some cells formed acinar or palisade clusters (Fig. 3b). In these cases, the list of differential diagnoses included hepatitis, hepatocellular adenoma, and nodular hyperplasia since the cytological picture did not allow an accurate diagnosis without additional methods of morphological diagnosis.

The histological examination confirmed the suspected diagnosis of hepatocellular carcinoma. Morphologically, this type of tumor has several developmental patterns: trabecular, pseudoglandular, solid, and scyrotic [4,25]. In 17/18 dogs, we observed the trabecular form of hepatocellular cancer, characterized by the formation of irregular-shaped trabeculae, which are separated by increased sinusoids and

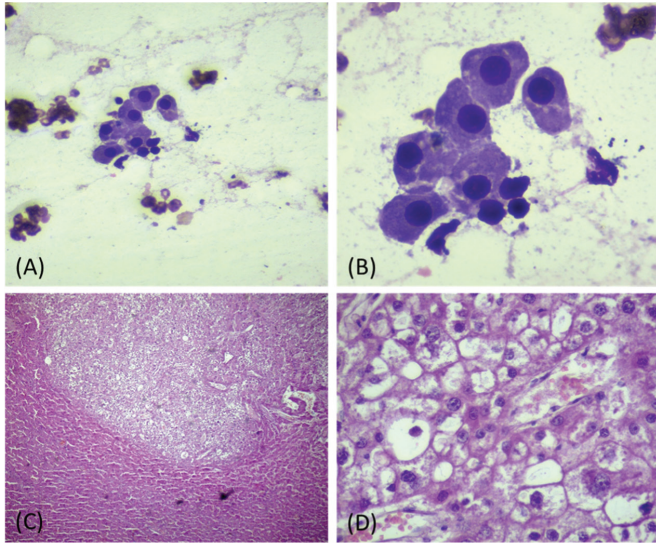


Figure 3. (A) Cytological picture of hepatocytes with minimal signs of malignancy, in the preparation of the dog with a histologically confirmed hepatocellular carcinoma. 100×, coloring according to Romanowsky. (B) The cytology preparation in the dog with hepatocellular cancer reveals a weak anisocytosis and anisokaryosis. 400×, coloring according to Romanowsky. (C) Histological picture of hepatocellular cancer. Tumor has abnormal lobule structure and is made up of hepatocytes forming solid layers. 10×, Hematoxylin-Eosin. (D) Histological picture of hepatocellular cancer. Cells have abundant eosinophilic cytoplasm with pronounced vacuolization. Nuclei are round and have 1–3 nucleoli. Anisocytosis and anisokaryosis are expressed moderately. 40×, Hematoxylin-Eosin.

spaces, and filled with blood or serous fluid. Trabeculae can have different widths depending on their location (Fig. 3c). A characteristic feature of this form of hepatocellular cancer is the formation of trabeculae with a thickness of 5–10 cm and more than 20 hepatocytes. The formation of such structures of varying thickness is one of the criteria for the differentiation of hepatocellular adenoma from adenocarcinoma. Tumor cells have a polygonal shape with an average nuclear-cytoplasmic ratio, eosinophilic cytoplasm, as well as central and paracentral nuclei with clearly visualized nucleoli (Fig. 3d). Some hepatocytes in this tumor may show pronounced vacuolization of the cytoplasm, due to the presence of glycogen or lipids in the tumor [21,25,28,29]. Similarly, in pleomorphic hepatocellular carcinomas, the cellular characteristics of hepatocyte malignancies are pronounced: the presence of pleomorphic cells, nuclei, and nucleolus, up to the formation of giant tumor cells [4]. Besides, hepatocellular carcinoma is characterized by the absence or presence of a low amount of connective tissue stroma [20,30].

The median survival of dogs included in this study was 234 days, with only 8/18 dogs surviving this period. Under

our supervision, two dogs have survived to date, aged 720 and 870 days. Only 5/18 dogs with a tumor size exceeding 5 cm had a lifespan of fewer than 100 days (30, 51, and 91 days).

Conclusion

Hepatocellular carcinomas have non-specific clinical manifestations, a characteristic aspect of other tumors of the hepatobiliary system. Methods of instrumental diagnostics (radiographic and ultrasound studies) are used to determine the location, size, and involvement of individual structures of the liver or other organs in the tumor process. Hematological changes in dogs with this pathology affect their final prognosis; however, the biochemical abnormalities caused by this pathology primarily reflect the changes in liver activity, not only indicating a specific pathology but also instead changes in the activity of AST, ALT, and ALP. An increased activity of AST, ALT, and ALP is considered as unfavorable prognostic signs for this pathology in canine species. In this study, all subjects showed an increased activity of at least one liver enzyme indicator, suggesting severe damage to the hepatocytes or aggressive tumor behavior, and indicating a lower survival prognosis. For instance, 5/7 dogs with a tumor size > 5 cm had a life expectancy of 30, 51, and 91 days, suggesting that the size of the tumor is an adverse prognostic factor.

Conflict of interests

We declare that authors have no competing interests.

Authors' contributions

Yury Vatinikov and Ilya Vilkovovskiy designed the experiment and coordinated all research activities; Evgeny Kulikov supervised the clinical work. Irina Popova, Nadia Khairova, and Aleksey Gazin performed the entire experiment; Andrey Zharov and Darya Lukina took part in preparing and critical evaluation of this manuscript. The final version was approved after all authors read the manuscript thoroughly.

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