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Evaluation of PIVKA-II and Histological Grading Systems as Prognostic Indicators in 22 Surgically Treated Canine Hepatocellular Carcinomas

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ABSTRACT

Hepatocellular carcinoma (HCC) in dogs is relatively uncommon and generally linked to a favorable outcome, although a subset of cases exhibits aggressive behavior. In human oncology, HCC typically presents as a highly malignant neoplasm, and various diagnostic and prognostic indicators—such as the expression of PIVKA-II—are used to assess tumor biology. To explore potential prognostic factors for canine HCC, several histological grading systems were applied, and PIVKA-II immunoreactivity was assessed in 22 surgically treated canine HCCs with a minimum clinical follow-up of two years. Among the studied cases, 19 dogs completed the observation period without recurrence, whereas 3 developed metastatic disease and died. Fifteen of 22 tumors showed positive PIVKA-II staining, but no clear relationship was identified between PIVKA-II expression, prognosis, or histologic grade, although a tendency toward PIVKA-II negativity in low WHO grades and positivity in higher grades was noted. The findings suggest that PIVKA-II cannot be regarded as a malignancy or prognostic marker in canine HCC. Clinical status at presentation remains the main determinant of outcome. To date, histopathological parameters capable of predicting tumor aggressiveness in canine HCC remain undefined. Within this investigation, the WHO histological grading appeared to be the most applicable system.

Keywords: PIVKA-II, Canine hepatocellular carcinoma, Prognostic indicators, WHO histological grading

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Introduction

Primary hepatic neoplasms account for less than 1.5% of all canine tumors [1]. Malignant hepatic lesions include hepatocellular carcinoma (HCC), cholangiocellular carcinoma, neuroendocrine tumors, and sarcomas, with HCC representing the most prevalent type—constituting 50–77% of all primary hepatobiliary malignancies in dogs [2]. HCC originates from hepatocytes, predominantly affects older animals, and shows no confirmed sex predisposition, though males may be slightly overrepresented [2, 3].

At present, canine HCC is not widely regarded as a comparative model in oncology. Unlike the human disease, the canine form is less frequent and usually less aggressive, particularly when presenting as the massive variant [2]. Additionally, while grading systems for HCC are well established in human medicine [4], such methods are rarely applied in veterinary pathology.

Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II) arises under vitamin K deficiency or exposure to vitamin K antagonists (e.g., warfarin). First identified by Liebman *et al*. (1984) as a potential tumor biomarker for HCC [5], subsequent human studies confirmed its clinical relevance and its association with HCC prognosis [6–8]. PIVKA-II is also used as a retrospective marker of vitamin K activity [9]. Elevated serum

PIVKA-II levels have been correlated with coagulation disorders and several neoplastic conditions, particularly HCC [10–12].

Immunohistochemical studies in humans revealed that PIVKA-II is expressed in small or well-differentiated tumor cells but not in adenomatous hyperplasia, supporting its diagnostic value in HCC [13]. Further investigations demonstrated a possible correlation between PIVKA-II expression and histologic grade in human HCC through analyses of formalin-fixed, paraffin-embedded tissues [14].

Our previous research identified PIVKA-II expression in canine liver and kidney, suggesting its role as a marker for coagulopathies or anticoagulant ingestion [15]. Despite its recognized prognostic significance in human oncology, PIVKA-II has not yet been evaluated for this purpose in animal tumors. Hence, the present study aimed to assess PIVKA-II expression in canine hepatocellular carcinoma and analyze its possible prognostic value based on clinical follow-up. Additionally, considering the absence of an established grading scheme for canine HCC, we sought to apply a recognized human histological grading system to determine its utility for prognosis prediction and relationship to PIVKA-II expression.

Materials and Methods

Sample collection and clinical follow-up

Tissue samples were obtained from 22 spontaneous cases of canine hepatocellular carcinoma surgically treated between 2005 and 2021 at the Department of Veterinary Sciences, University of Turin. None of the dogs exhibited macroscopic metastases at diagnosis, as confirmed by whole-body CT or abdominal ultrasound with three-view thoracic radiographs. All cases underwent complete surgical excision of the hepatic mass.

Postoperative clinical assessments, abdominal ultrasonography, and thoracic radiographs were scheduled every three months during the first year and every six months in the second year. Long-term data were gathered via owner telephone interviews. Dogs surviving beyond two years post-surgery were censored for statistical analysis. For those who died from tumor-related causes within two years, overall survival (OS) was calculated from surgery to death, and the disease-free interval (DFI) represented the duration from surgery to recurrence or metastasis.

Histological examination

Tumor specimens were fixed in 4% neutral-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). Two independent pathologists (L.M. and K.V.) graded the samples based on nuclear pleomorphism, nucleolar variability, and architectural features, following criteria established by both the World Health Organization (WHO) [16] and Martins-Filho *et al*. (2017) **(Table 1)** [4].

Table 1. Histological grading criteria according to Martins-Filho *et al*. (2017) [4].

Nuclear Grade	**Description**
I	Uniform nuclei resembling normal
II	Slight variation in nuclear size and shape
III	Moderate variation in nuclear size and shape, uneven chromatin distribution
IV	Severe variation in nuclear size and shape, abnormal nuclei
Nucleolar Grade	**Description**
I	Nucleoli scarcely visible at 400× magnification
II	Nucleoli clearly visible at 100–200× magnification
III	Prominent nucleoli visible at 100× magnification
IV	Large nucleoli visible at 40× magnification
Architectural Grade	**Description**
I	Trabecular pattern, 2–3 cells thick
II	Pseudoglandular arrangement
III	Mild trabecular pattern (4–10 cells thick)
IV	Macro-trabecular (>10 cells thick) or solid with abnormal patterns

Immunohistochemistry

Immunohistochemical evaluation for PIVKA-II expression was carried out following the previously established protocol [15]. The procedure utilized a rabbit polyclonal anti-PIVKA-II antibody (1:2000 dilution; custom-produced by Diatheva Srl, Cartoceto, Italy). For the negative control, sections were incubated with the immunoglobulin fraction from non-immune mouse serum in place of the primary antibody. The stained slides were independently examined under a light microscope by two blinded pathologists (L.M. and K.V.), who were unaware of the corresponding clinical data and histological classifications.

Statistical analysis

Associations between IHC outcomes and clinicopathologic parameters were organized in contingency tables and statistically tested using Fisher's exact test or the χ^2 test. Overall survival (OS) and disease-free interval (DFI) were analyzed using the Kaplan–Meier estimator, and differences between groups were determined through the log-rank test. All computations were conducted using MedCalc Statistical Software version 13.3 (MedCalc Software bvba, Ostend, Belgium).

Results and Discussion

Clinico-pathological findings

A total of 22 canine cases of hepatocellular carcinoma were included. Both sexes were evenly represented. A summary of all clinical and histological data is provided in **Table 2**. The majority of dogs (17/22; 77.27%) completed the observation period without tumor recurrence. Two dogs (13.64%) succumbed shortly after surgery due to operative complications, while three dogs developed metastatic disease and died from tumor-related causes.

Table 2. Signalment (breed, age, sex), histologic grades, immunohistochemical outcomes, and clinical follow-up data.

Case No.	Breed	Age (years)	Sex	Nuclear Grade	Nucleolar Grade	Architectural Grade	WHO Grade	PIVKA-II	DFI (days)	OS (days)	Notes
1	Samoyed	10	M	2	4	4	4	1	35	60	Metastases
2	Yorkshire Terrier	13	FS	1	2	2	2	1	730	730	*
3	Beagle	12	M	1	1	1	1	0	730	730	*
4	Mixed Breed	10	FS	3	3	2	3	0	1	1	Death due to surgical complications
5	Golden Retriever	10	M	1	1	4	3	0	730	730	*
6	Dachshund	7	M	1	1	1	1	0	730	730	*
7	Mixed Breed	13	FS	1	1	4	3	0	730	730	*
8	Mixed Breed	8	M	1	1	2	2	0	730	730	*
9	Mixed Breed	9	FS	2	3	2	2	1	730	730	*
10	Mixed Breed	13.5	M	2	3	2	2	1	730	730	*
11	Mixed Breed	12	M	3	4	4	4	1	365	365	*
12	Cocker Spaniel	10	F	1	2	2	2	1	730	730	Relapsed at 900 days but alive
13	Mixed Breed	11	FS	2	1	3	3	1	365	365	Metastases
14	Siberian Husky	13	FS	2	4	2	3	1	5	5	Death due to surgical complications
15	Cocker Spaniel	11	FS	1	3	1	2	1	365	365	*
16	Golden Retriever	8	M	1	3	3	2	1	730	730	*

17	Schnauzer	13	M	1	2	2	2	1	730	730	*
18	Golden Retriever	10	M	2	4	3	3	1	730	730	*
19	Mixed Breed	12	FS	4	4	4	4	1	730	730	*
20	Rottweiler	12	MC	1	2	3	3	0	30	30	Metastases
21	Yorkshire Terrier	13	FS	2	4	1	2	1	730	730	*
22	Mixed Breed	13	FS	2	1	1	2	1	730	730	*

*Censored cases represent dogs alive ≥2 years post-surgery, free from local recurrence or metastasis, or deceased from non-tumor-related causes.

The application of histological grading according to Martins-Filho *et al*. (2017) [4] revealed marked variation in grading outcomes depending on whether architectural, nuclear, or nucleolar morphology was considered **(Table 2)**. Cytoplasmic PIVKA-II immunoreactivity was observed in tumor cells, with weak positivity consistently seen in adjacent non-neoplastic hepatic tissue **(Figure 1)**. Out of the 22 total samples, 15 (68.18%) showed PIVKA-II positivity, while 7 (31.82%) were negative.

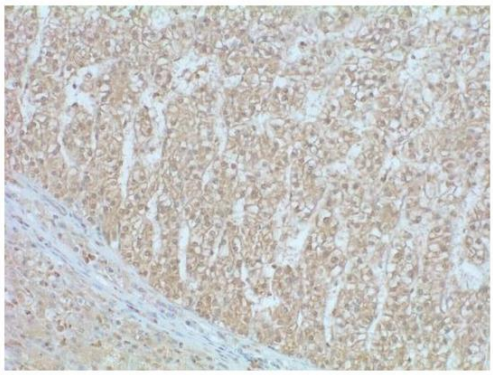

Figure 1. Well-differentiated hepatocellular carcinoma (WHO grade) with a trabecular pattern demonstrating positive PIVKA-II cytoplasmic staining. The peritumoral hepatic region (lower left) shows mild PIVKA-II expression, whereas the fibrous capsule is negative. Magnification: 200×; counterstain: Mayer's hematoxylin.

Statistical analysis

No significant correlations were found between PIVKA-II immunoreactivity and clinical or pathological variables overall. However, within the WHO-based grading system, a statistical association was detected between grade 2 tumors and PIVKA-II positivity ($p < 0.05$). A tendency for PIVKA-II negativity in lower WHO grades and increased positivity in higher grades was also observed **(Figure 2)**.

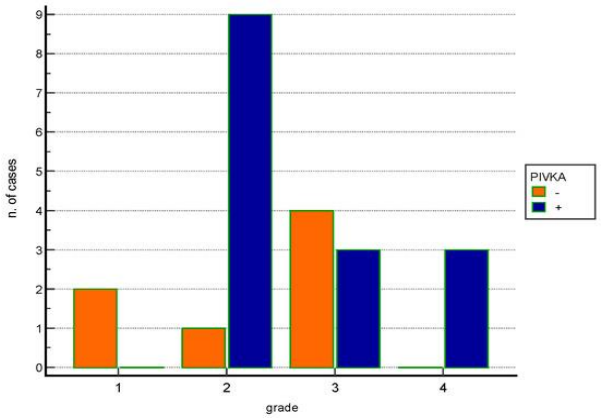

Figure 2. Histogram illustrating PIVKA-II positive vs. negative cases across WHO histological grades.

Further analysis of the alternative grading parameters revealed a significant association between nucleolar pleomorphism grade 4 and PIVKA-II positivity (Fisher's Exact test, $p < 0.05$) **(Figure 3).**

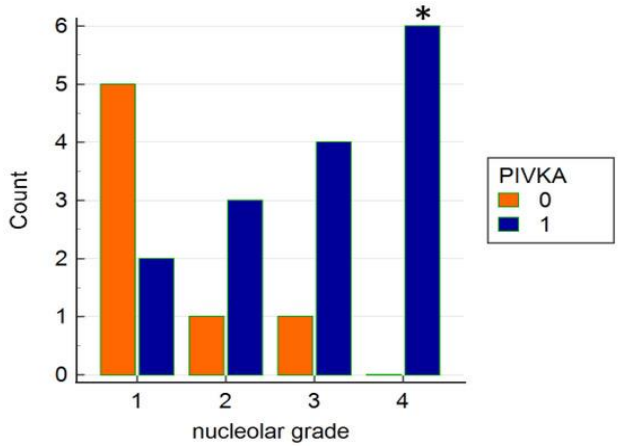

Figure 3. Histogram showing PIVKA-II expression relative to nucleolar pleomorphism grades. * Indicates Fisher's Exact test, $p < 0.05$.

The log-rank tests comparing IHC data with OS and DFI demonstrated no significant relationships. Additionally, evaluation of survival in relation to histologic grading identified a significant correlation between nuclear pleomorphism grade 3 and shortened survival, though this trend was not maintained for grade 4 tumors. No other statistically meaningful associations between survival outcomes and the parameters analyzed were found.

To the best of the authors' knowledge, PIVKA-II immunoreactivity in canine hepatocellular carcinoma (HCC) has not been previously examined. PIVKA-II represents an abnormal variant of prothrombin produced by hepatic cells under vitamin K deficiency, which can occur during coagulation abnormalities, after vitamin K antagonist exposure [17, 18], or in certain neoplastic conditions where it serves as a prognostic indicator in human HCC [19–21]. This atypical prothrombin has also been employed to assess coagulopathies in dogs. Indeed, the PIVKA-II plasma assay was reported by Mount *et al*. as a diagnostic aid for differentiating anticoagulant intoxication from other bleeding disorders in canines [22].

In earlier work, we showed that PIVKA-II can be expressed in both hepatic and renal tissues of dogs that died due to anticoagulant toxicosis, as well as in animals affected by coagulative disturbances or hepatic degeneration [15]. In the present investigation, PIVKA-II expression in canine HCC appeared variable, yet the adjacent non-neoplastic hepatic parenchyma consistently displayed weak immunopositivity. This observation corresponds with our prior findings, where degenerating hepatocytes exhibited PIVKA-II expression, most likely reflecting secondary coagulopathy due to liver dysfunction [15].

It may be hypothesized that, under neoplastic compression, the surrounding liver tissue, even when histologically degenerated, undergoes altered coagulation metabolism, resulting in PIVKA-II synthesis. However, we could not substantiate this assumption by correlating with serum coagulation profiles, as such data were unavailable. Furthermore, given that these dogs were surgical candidates, it is plausible that their coagulation indices remained largely within normal limits.

Our findings contrast with certain human studies, in which PIVKA-II was described as a specific indicator of malignancy, showing strong positivity in neoplastic hepatocytes but negative staining in surrounding regenerative or cirrhotic tissue [13, 23]. Because PIVKA-II did not perform as a discriminative tumor marker in canine liver neoplasms, we assessed whether its expression might correlate with the histologic differentiation of HCC.

In dogs, HCC typically exhibits low malignancy, and a standardized histological grading system is not established in veterinary pathology. Therefore, we applied grading frameworks commonly used in human HCC, including the WHO classification, as well as systems based on architectural patterns, nuclear morphology, and nucleolar pleomorphism.

As summarized in **Table 1**, no consistent correlation was found among these grading criteria, showing marked variability. For instance, in cases 4 and 7, nuclear and nucleolar pleomorphism corresponded to low-grade features, whereas tumor architecture indicated high-grade malignancy. Within the limitations of our sample size,

the WHO grading method, as adapted by Martins-Filho *et al*. for human HCC, appeared the most applicable to veterinary specimens.

Although no statistically significant link was observed between histologic grade and survival duration, we cannot dismiss the possibility that a larger cohort might reveal such an association. The absence of clear results in this study may reflect the limited sample size, the number of censored cases, and the fact that only a few dogs with metastases exhibited WHO grade 3 or 4 tumors.

Conclusion

Within the limits of the current dataset, PIVKA-II does not appear to function as a diagnostic or prognostic marker for canine HCC, differing from its recognized role in human hepatic carcinoma. This outcome requires confirmation through larger investigations.

There remains a clear need for reliable prognostic indicators capable of predicting aggressive biological behavior in canine HCC, which, although uncommon, can occur in some cases. Among the evaluated parameters, the WHO histologic grading scheme emerged as the most promising tool, but it likewise warrants validation in a broader cohort to confirm its prognostic significance.

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References

1. Liptak J, Dernell W, Monnet E, Powers B, Bachand A, Kenney J, et al. Massive hepatocellular carcinoma in dogs: 48 cases (1992–2002). J Am Vet Med Assoc. 2004;225(8):1225–30.
2. Gibson EA, Goldman RE, Culp WTN. Comparative oncology: Management of hepatic neoplasia in humans and dogs. Vet Sci. 2022;9(11):489.
3. Kinsey JR, Gilson SD, Hauptman J, Mehler SJ, May LR. Factors associated with long-term survival in dogs undergoing liver lobectomy as treatment for liver tumors. Can Vet J. 2015;56(6):598–604.
4. Martins-Filho SN, Paiva C, Azevedo RS, Alves VAF. Histological grading of hepatocellular carcinoma: A systematic review. Front Med. 2017;4:193.
5. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, et al. Des-gamma-carboxy prothrombin as a serum marker of primary hepatocellular carcinoma. N Engl J Med. 1984;310(22):1427–31.
6. Dong R, Wang N, Yang Y, Ma L, Du Q, Zhang W, et al. Review on vitamin K deficiency and its biomarkers: Focus on PIVKA-II. Clin Lab. 2018;64(3):413–24.
7. Feng H, Li B, Li Z, Wei Q, Ren L. PIVKA-II complements AFP for diagnosis of hepatocellular carcinoma. BMC Cancer. 2021;21(1):401.
8. Huang S, Jiang F, Wang Y, Yu Y, Ren S, Wang X, et al. Diagnostic performance of AFP and PIVKA-II in Chinese hepatocellular carcinoma patients. Tumour Biol. 2017;39(4):1010428317705763.
9. Card DJ, Gorska R, Harrington DJ. Laboratory assessment of vitamin K status. J Clin Pathol. 2020;73(2):70–5.
10. Kim DY, Paik YH, Ahn SH, Youn YJ, Choi JW, Kim JK, et al. PIVKA-II as a tumor marker for recurrent hepatocellular carcinoma after resection. Oncology. 2007;72(Suppl 1):52–7.
11. Kim JM, Kwon CH, Joh JW, Park JB, Lee JH, Kim SJ, et al. PIVKA-II as a useful marker in modified UICC T3 hepatocellular carcinoma. Hepatogastroenterology. 2013;60(126):1456–2.
12. Kim JM, Hyuck C, Kwon D, Joh JW, Lee JH, Paik SW, et al. Protein induced by vitamin K antagonist-II (PIVKA-II) as a prognostic factor in small hepatocellular carcinoma. World J Surg. 2013;37(6):1371–8.
13. Miskad UA, Yano Y, Nakaji M, Kishi S, Itoh H, Kim SR, et al. Histological study of PIVKA-II expression in hepatocellular carcinoma and adenomatous hyperplasia. Pathol Int. 2001;51(12):916–22.

14. Tamano M, Sugaya H, Oguma M, Murohisa T, Tomita Y, Matsumura A, et al. Immunolocalisation of PIVKA-II in hepatocellular carcinoma. Liver. 1999;19(5):406–10.
15. Maniscalco L, Varello K, Zoppi S, Abbamonte G, Ferrero M, Torres E, et al. Abnormal PIVKA-II expression in canine tissues as an indicator of anticoagulant poisoning. Animals. 2021;11(9):2612.
16. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. WHO classification of digestive system tumours (2019). Histopathology. 2020;76(2):182–8.
17. Brenner B, Kuperman AA, Watzka M, Oldenburg J. Vitamin K-dependent coagulation factor deficiency. Semin Thromb Hemost. 2009;35(5):439–46.
18. Ajisaka H, Shimizu K, Miwa K. Immunohistochemical study of PIVKA-II in hepatocellular carcinoma. J Surg Oncol. 2003;84(2):89–93.
19. Arnesen H, Smith P. Predictability of bleeding by PIVKA-sensitive and insensitive prothrombin times. Thromb Res. 1991;61(3):311–4.
20. Basile U, Miele L, Napodano C, Ciasca G, Gulli F, Pocino K, et al. Diagnostic performance of PIVKA-II in metabolic and viral HCC. Eur Rev Med Pharmacol Sci. 2020;24(22):12675–85.
21. Heckemann HJ, Ruby C, Rossner K. Determination of coagulation factors and PIVKA inhibitors. Folia Haematol Int Mag Klin Morphol Blutforsch. 1988;115(4):533–7.
22. Mount ME, Kim BU, Kass PH. Use of PIVKA test in diagnosis of anticoagulant poisoning in dogs: 325 cases (1987–1997). J Am Vet Med Assoc. 2003;222(2):194–8.
23. Lim TS, Kim DY, Han KH, Kim HS, Shin SH, Jung KS, et al. Combined use of AFP, PIVKA-II and AFP-L3 improves diagnostic accuracy for hepatocellular carcinoma. Scand J Gastroenterol. 2016;51(3):344–53.