PhD THESIS

The clinicopathological significance of certain molecular markers in the diagnosis and prognosis of gastrointestinal tumors in dogs and cats

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INTRODUCTION

Gastrointestinal tumors account for more than a quarter of all neoplastic formations that can develop in humans (Sung et al., 2021). In recent years, a concerning increase in the global incidence of these malignancies has been observed, with approximately 2 million new diagnoses and around 900,000 deaths reported annually (Xi et al., 2021; Morgan et al., 2022). An alarming aspect is the rising number of young patients diagnosed with these neoplasms worldwide (Vuik et al., 2019). In Romania, according to a study conducted in 2018 and published in 2021, the number of new colorectal cancer cases was 14,085, with a continuous increasing trend (Ionescu et al., 2021). Although the use of laboratory animals provides significant advantages in human cancer research, the success rate of translating findings from animal models to humans in clinical oncology trials is approximately 8% (Marck et al., 2014).

Gastrointestinal tumors in dogs and cats are rare compared to those diagnosed in humans, accounting for less than 1% of cases. However, numerous similarities exist between neoplasms in these species and human malignancies, particularly in epithelial tumors. These similarities are observed at both the microscopic and molecular levels, including genetic mutations such as *KRAS, CTNNB1, TP53,* and *EGFR*, which are involved in carcinogenesis (Groll et al., 2021). Furthermore, significant parallels have been identified in lymphomas affecting both dogs and humans, in terms of clinical presentation, tumor biology, neoplastic behavior, and the presence of common genetic aberrations (Marconato et al., 2013).

Given the challenges and ethical implications associated with using dogs and cats in experimental research, a promising alternative for studying gastrointestinal tumors is the development of cell lines derived from neoplasms diagnosed in these species. One example is the FeLeco-G7 cell line, derived from a feline colorectal adenocarcinoma, which has been successfully xenotransplanted into mice, yielding promising results (Uneyama et al., 2024).

Considering the significant global impact of gastrointestinal tumors and their high mortality rate, it is crucial to identify novel diagnostic and prognostic markers and to develop innovative therapeutic strategies.

THE STRUCTURE OF THE THESIS

The thesis entitled "The clinicopathological significance of certain molecular markers in the diagnosis and prognosis of gastrointestinal tumors in dogs and cats" contains 119 pages and is written in accordance with current academic standards. It is structured into two main parts.

The first part, which represents the review of the literature, is organized into three chapters and spans 16 pages. This part synthesizes the current knowledge ramework the epidemiology of major gastrointestinal tumors in companion animals, the diagnostic techniques, including clinical presentation, imaging methods, cytological, histological, and immunohistochemical analyses, as well as micro-RNA-based investigations and oncogenesis mechanisms.

The second section presents the personal research conducted between 2021 and 2025, extending over 70 pages and structured into four chapters. Each chapter is divided into sub-chapters detailing the study's aims and objectives, the materials and methods employed, the results obtained, and a comparative analysis with existing studies. Additionally, each study concludes with relevant conclusions.

The research findings are illustrated through 34 figures and summarized in 11 tables. The paper concludes with a cited bibliography comprising 236 references.

RESULTS OF RESEARCH

In the second part of this work, we investigated the identification of new immunohistochemical markers with potential utility in the diagnosis and prognosis of gastrointestinal tumors in dogs and cats. The main objectives we set out to achieve were:

- Conducting a retrospective, interinstitutional epidemiological study across six countries on neoplastic and non-neoplastic proliferative gastrointestinal lesions in dogs and cats.
- Analyzing BMP-2 expression in canine intestinal osteosarcoma using immunohistochemical techniques.
- Characterizing the immunohistochemical profile of follicular gastritis in dogs and its correlation with helicobacter colonization and canine gastric lymphoma.
- Assessing the expression of 14-3-3σ in epithelial intestinal tumors in dogs and cats and evaluating its diagnostic and prognostic potential.

Chapter 5, titled "Non-neoplastic and neoplastic proliferative lesions of the gastrointestinal tract in dogs and cats: interinstitutional epidemiological study and morphological characterization." aimed to conduct an interinstitutional epidemiological study by identifying non-neoplastic proliferative lesions and neoplastic formations localized in the gastrointestinal tract of dogs and cats. Epidemiological data were retrospectively collected from universities and private laboratories databases across six countries—Romania, Brazil, Portugal, Poland, Russia, and Israel—covering a period of 10 years. A total of 475 cases of neoplastic and non-neoplastic proliferative lesions were selected and subjected to statistical analysis. Elderly animals were more predisposed to chronic hypertrophic pyloric gastropathy (CHPG) (mean age: 11.2 years) and gastric polyps (mean age: 9.6 years), whereas younger individuals exhibited a higher incidence of feline gastrointestinal eosinophilic sclerosing fibroplasia (FGIESF), with a mean age of 7.25 years for stomach lesions and 5.25 years for intestinal lesions. Additionally, CHPG and FGIESF were

more frequently diagnosed in males. Among gastrointestinal tract tumors, malignant neoplasms accounted for 88% of cases. Lymphomas had an incidence of 38.74%, while adenocarcinomas represented 27.16% of cases. In cats, lymphomas were the most frequently diagnosed tumors, with 141 cases reported. In contrast, dogs were the primary species affected by benign neoplasms, including leiomyomas (58.62%) and adenomas (42.10%). Regarding malignant neoplasms, dogs were more predisposed to developing adenocarcinomas (92 cases), which accounted for 39% of all malignant tumors, followed by lymphomas (12%) and gastrointestinal stromal tumors (GIST) (9%). Regardless of species, the small intestine was the most common localization of gastrointestinal lesions.

Chapter 6, titled "Molecular Markers Used in the Diagnosis and Prognosis of Rare Gastrointestinal Tumors in Dogs and Cats: Intestinal Osteosarcoma" aimed to present a case of intestinal osteosarcoma, potentially induced by the recurrent ingestion of cotton fragments from various textile materials. To investigate this case, the clinical condition of the patient was assessed, including biochemical, hematological, and imaging examinations (ultrasound). During exploratory laparotomy, tissue samples were collected in 10% formalin for histopathological and immunohistochemical examination. Histopathological analysis revealed a transmural neoplastic mass in the jejunum, ulcerated on the surface and composed of cells arranged in bundles. Multifocally, an eosinophilic, acellular material interpreted as osteoid was identified, confirmed through Masson's Trichrome staining. Immunohistochemically, neoplastic cells exhibited moderate cytoplasmic expression for Bmp-2, while multinucleated neoplastic cells showed intense positivity for the same antibody. Additionally, the clinical progression of the patient was monitored, revealing hepatic metastases six months post-surgery.

Chapter 7, titled "Follicular gastritis (gastric lymphofollicular hyperplasia) in dogs: pathological and immunohistochemical features"had the nain objective to evaluate and characterize follicular gastritis (FG) in dogs from an immunohistochemical perspective. To achieve this, the following objectives were set: assessment of endoscopic lesions and clinical manifestations in dogs diagnosed with FG, evaluation of *Helicobacter* spp. colonization and the associated inflammation in follicular gastritis, immunohistochemical characterization of lymphocyte distribution within lymphoid follicles and comparison with gastric lymphomas using CD3, Pax5, and Bcl6 antibodies, and correlation of follicular gastritis with gastric mucosal alterations and *Helicobacter* spp. colonization. The study analyzed 41 cases of FG in dogs. Young dogs (mean age: 3.52 years), particularly French Bulldogs (75.06%), were more predisposed to FG. Endoscopic findings included lymphoid nodules in both the gastric antrum and corpus, while gastric ulcers were observed in 9 out of 41 cases. The diameters of lymphoid follicles were similar in the gastric corpus (mean: 295.587 µm) and antrum (mean: 294.641 µm). Histopathologically, GF-associated lesions included glandular atrophy, which was more common in the antral region (10 out of 17 cases), lymphoplasmacytic inflammation, and fibrosis. *Helicobacter* spp. colonization was classified as mild, moderate, or severe in 20, 6, and 3 cases, respectively. In cases of mild colonization, spiral bacteria were identified only on the mucosal surface, whereas in severe infections, bacteria were also observed in the gastric crypts and within the cytoplasm of parietal cells. Immunohistochemically, B-lymphocytes positive for Bcl6 and Pax5 were centrally located in large follicles, surrounded by CD3+ T-lymphocytes. Small follicles lacked germinal centers, showing no Bcl6 immunostaining, but contained both CD3+ T-lymphocytes and Pax5+ B-lymphocytes. For comparison, two T-cell and two B-cell lymphomas were analyzed. In both lymphoma types, immunostaining was diffuse, with no follicular structures. T-cell lymphomas were CD3-positive, with multifocal rare Bcl6+ and Pax5+ B-lymphocytes. B-cell lymphomas were Pax5-positive and CD3-negative, with a significant number of Bcl6-positive lymphocytes.

A positive correlation was found between follicle diameter and Helicobacter spp. colonization ($p = 0.049$) and follicular hyperplasia ($p < 0.001$). A regression analysis indicated that *Helicobacter* spp. colonization and hyperplasia accounted for 42.3% of follicle diameter variation ($R^2 = 0.423$, $p < 0.001$).

Chapter 8, is titled "The clinicopathological significance of the 14-3-3σ protein expression in intestinal epithelial tumors in dogs and cats". The study aimed to evaluate the expression of 14-3-3σ protein in intestinal tumors of dogs and cats and determine its potential as a diagnostic and prognostic marker. The objectives included: retrospective analysis of gastrointestinal epithelial tumors, considering clinico-pathological data (species, breed, age, sex). Histological classification of tumor formations following World Health Organization (WHO) standards, including tumor staging and invasion analysis. Evaluation of 14-3-3σ expression in intestinal tumors using immunohistochemical methods and digital quantification. The study included 43 cases of intestinal tumors, comprising 35 cases in dogs (16 adenomas and 19 adenocarcinomas) and 8 adenocarcinomas in cats. Adenomas were exclusively located in the rectum. Histopathologically, the papillary subtype was the most frequent in adenomas (62.5%), while papillary adenocarcinomas (47.36%) were more common in dogs, whereas the tubular subtype was more frequently diagnosed in cats (75%). Tumor staging revealed that in dogs, grade 1 adenocarcinomas were most prevalent (52.63%), while in cats, grade 4 adenocarcinomas were most common (63%), with no grade 1 cases observed.

Immunohistochemically, positive staining for 14-3-3σ was detected in 62.96% of adenocarcinomas and 56.25% of adenomas. In dogs, 57.89% of adenocarcinomas exhibited positive staining, with moderate intensity in most cases. In cats, all 8 adenocarcinomas showed cytoplasmic immunostaining, predominantly of moderate intensity. Histologically, 14-3-3σ was expressed in most tubular and papillary adenocarcinomas, as well as in one anaplastic case. Sporadic nuclear immunostaining was observed, particularly in association with metastases and emboli. In dogs, no statistically significant differences were found between the mitotic figure count and the percentage of stained area. In contrast, in cats, adenocarcinomas displayed high proliferative activity, independent of marker expression ($p < 0.05$), suggesting aggressive behavior. Additionally, in dogs, the marked surface area is significantly larger than tumor infiltration into histological layers, suggesting that the expression of the 14-3-3σ protein occurs early, prior to deep tumor infiltration. In contrast, in cats, although there is a correlation between the marked surface area and the degree of tumor invasion, this correlation is weaker, indicating a potential decrease in protein expression as the disease progresses.

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