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EPIDERMAL GROWTH FACTOR RECEPTOR OVER-EXPRESSION IN CANINE GASTRIC ADENOCARCINOMA IN 36 DOGS

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Abstract

Gastric adenocarcinoma is the most frequent tumour which affects the stomach of dogs, having multiple causes. EGFR (Epidermal Growth Factor Receptor) has an important impact in the development and expansion of gastric tumours, representing the first receptor which provides the connection between overexpression and cancer. The present study included 36 dogs with digestive syndromes, expressed as vomiting and melaena. Routine histopathological examinations on full-thickness gastric biopsies sampled by endoscopy, and on the tissue samples taken during necropsy, were completed by EGFR identification by immunohistochemistry, which could be used to estimate prognosis and select therapy. It has been concluded that 14 cases presented signet-ring cell adenocarcinoma, nine patients with tubular adenocarcinoma, ten with papillary adenocarcinoma, while the rest of three patients had undifferentiated adenocarcinoma. The highest rates of EGFR expression were identified in 38.4% of cases (n=10), highlighting a worse prognosis. EGFR expression was associated with the location of tumoural type, tumour size, cell differentiation, invasion, being positive in 26 cases of gastric cancer tissue (72%), while in the rest of ten cases (28%) were featured by low expression.

Key words*: EGFR, gastric adenocarcinoma, gastritis.*

INTRODUCTION

Gastric adenocarcinoma (GA) is the most frequent tumour with epithelial origin, representing between 50-90% of gastric tumours in dogs (Patnaik A.K., 1977; Swann H.M., 2002). Usually, the aetiology of neoplasia remains uncertain, but in additional instances it may be represented by the animal's diet, polyposis, *Helicobacter pylori* presence and ulcerative colitis. The prognosis of gastrointestinal adenocarcinoma in dogs varies according on the tumour site (Basil M., 1974) Mucous gastric atrophy, which is caused by *Helicobacter pylori* infection and observed at an early age population, contributes to the appearance of gastric cancer (Forman, 1991). In addition, a genetic mutation is considered also responsible in the transition from adenomatous polyposis and gastric adenoma to GA (Yoshiazaki K., 2020). The World Health Organization (WHO) defines stomach carcinomas in dogs based on their development arrangement, categorizing them into five distinct groups: tubular carcinoma, mucinous carcinoma, signet-ring cell carcinoma, undifferentiated carcinoma, and papillary carcinoma (Head K., 2003).

The studies concerning the association between gastric diseases and *Helicobacter pylori* infection have demonstrated that the existence of chronic inflammation accompanied by this bacterial infection cause the major changes of the gastric mucosa, stimulating epithelial cell proliferation, PH secretion, and suppression of cells apoptosis, as well as a development of stem cell niches, enhancing the incidence of stomach tumours (Geyer C., 1993; Hermanns, W.,1995; Yamasaki K., 1998; Asaka M., 2002).

The gastric corpus and the pyloric antrum have different stem cells and stem cells niches which reveals that cancer has two sites as origins, the most common anatomical location being represented by pyloric region (Hayakawa Y., 2017; Saito T., 2020). Certain breeds are more often predisposed to GA, being diagnosed with severe gastric-mucosal atypia, such as: Jack Russel Terrier, Golden Retriever, Australian Terrier, Belgian Tervuren, Cairn Terrier, Collie and Siberian Husky (Candido M.V., 2018) Some studies have demonstrated that a large percentage of Jack Russell Terriers have been diagnosed with gastric adenomas and adenocarcinoma, the most frequent site being the pylorus (Saito T., 2020; Yoshizaki K., 2020)

The significance of EGF in gastric cancer is associated with invasiveness of gastric wall and lymph node metastasis, featuring higher malignant potential. EGF is an essential regulator which generates the gastrointestinal tissue differentiation and proliferation of cells through its receptor, EGFR. EGFR plays a key role in establishing the messenger pathway which determines the proliferation, motility adhesion, survival time, and malignancies of gastric tumours (Tokunaga A., 1995; Woodburn J., 1999; Xia L., 2002)

Clinical signs, complementary blood exams, endoscopic and abdominal ultrasound assessments, macroscopic evaluations, cytological and histopathological investigations, EGFR test were all needed during various stages to distinguish the tumour from the inflammatory process and to minimize inaccurate diagnoses. It was necessary to evaluate the results, determining the essential criteria, correctly interpreting macroscopic, cytological, and histological features, and accurately determining EGFR outcomes.

The methods to diagnose gastric tumours are multiples, but the EGFR, as immunohistochemistry method in diagnosis of GA in dogs, discovered in 1961, is the most used method and it has a major target for gastric cancer diagnostic and therapy (Cohen S., 1962; Mishani E., 2008; Consolaro A., 2010) The prognostic and therapeutically protocols are based on the results of cytopathological and histopathological complementary examinations (Washabau R.J., 2010)

MATERIALS AND METHODS

Dogs' recruitment

A total of 36 dogs of different breeds were included in the present study, as follows: Collie (n=3), French Braque (n=4), Golden Retriever (n=4), Jack Russel Terriers (n=8), Belgian Tervuren (n=6), Swiss White Shepherd (n=6), Labrador (n=2), Fox Terrier (n=3). For all dogs, medical issues were typically identified at patients aged from 9 to 13 years old, independent of gender. A standard diagnostic protocol has been used for all cases, which consisted of: a history, physical examination, blood work tests (hemogram, biochemistry, serum cobalamin concentration, liver enzymes), complete endoscopy, abdominal ultrasound examination, macroscopical analysis of stomach (observed in necropsies and surgeries), cytological and histopathological examinations of all stomach regions collected from endoscopies and surgeries, *Helicobacter pylori* identification method by coloration with Haematoxylin-Eosin statement, immunohistochemistry staining for EGFR expression.

Blood examination

The blood has been collected on EDTA tubes using a differential white blood cell count (WBC) and serum tubes for biochemistry, including examination of serum bile acid concentration, serum vitamin B_{12} and folate, serum albumin concentration, on IDEX system. In 3 out 36 dogs, the lactate dehydrogenase was measured.

Endoscopy, abdominal ultrasound examinations and collection of samples

A preliminary diagnosis was achieved by performing a complete endoscopic examination with an 8 mm diameter frequency range flexible endoscope which allowed inspection of gastric mucosa and collection of upper gastric tract tissue samples, in order to establish histopathological diagnosis. Depending on the lesion's localization, endoscopic ultrasonography procedures used complete width resection and endoscopic submucosal excision. The gastric samples have been collected using an endoscope equipped with a full thickness resection device, a needle cap with 10 mm diameter over the scope clip, and a preloaded 12 mm electrosurgical catch.

Abdominal ultrasonography examination was performed in all patients, using a high-frequency linear transducer (15 MHz).

Standard necropsy procedure was carried out in 14 dogs dead or euthanized animals, and included gross assessment and tissue samples for microscopical investigation.

Gross lesions have been observed and analysed also during surgeries procedures such as gastrotomy (n=16), being followed by sampling for histopathological investigations.

Full thickness gastric biopsies, resections and endoscopic submucosal excision were performed on living animals (n=22) of different

gastric segments. Twelve patients had surgical removal of tumour gastric mass with 2-4 cm surgical safety margins.

Cytological examination

Cytological investigation was associated with histological and macroscopically analyses, having the role in identification of malignancy processes. Following FNA and full thickness gastric biopsies, the samples were air-dried and Diff-Quick stained.

Histological criteria

Histopathological examinations have been performed in all gastric samples (cardia, pyloric segments, fundus), applying a routine procedure of staining and examination. All specimens had 3 mm thick and were immersed in 10% neutral buffered formalin for two days, and finally coloured with haematoxylin and eosin staining.

The incidence of *Helicobacter pylori* has been determined in all 36 cases using the following procedures: ELISA test (by specific serological antibody method), PCR real time, in contrast to immune-histology examination of gastric mucosal samples obtained through endoscopy biopsies of greater, lesser curvature and fundus from living and from necropsied dogs, to relate these diagnostic approaches with pathology significance in dogs with this infection. Following PCR analysis of *Helicobacter* spp., the specific patterns after that 3% of electrophoresis in agarose gel was used, revealed positive results in n=15 from all tissues samples, in an immunoreactive band with approximately 500bp.

EGFR Immunohistochemistry method

The tissue specimens have been screened for EGFR test via an antigen with standardized immunohistochemistry sensitivity. FFPE tissues sections with 5 µm thickness were cleaned, rehydrated, and incubated in 4% hydrogen peroxide in combination with distilled water for 40 minutes.

All samples have been prepared with citrate buffered saline solution at 95°C for 20 min, and incubated with 0.5% hydrogen peroxide solution in methanol for a duration of 15 minutes.

All cases were treated with symptomatic treatments before complementary examinations and register of favourable responses. The symptomatic treatments included: (parasiticides, antibiotics, steroidal anti-inflammatory drug therapy, and gastro-intestinal protectants).

After a classification of responses at the symptomatic treatments, diagnostic results, and general evolution, the patients were treated as presented in Table

Surgery resection was applied in 12 cases, 3 patients had complete tumour resection, and 9 had partial tumour resection.

The potential prognosis was concluded in correlation with the evolution of each patient after the tumour, or partial tumour resection, chemotherapy treatments, localization of the tumour and in function of severity of diagnosis.

RESULTS AND DISCUSSIONS

The most common clinical signs in all patients have included: changes in appetite (n=30), chronical vomiting (n=28), diarrhoea (n=15), melaena (n=10), weight loss (n=25), haematochezia (n=13), hematemesis (n=16), polydipsia (n=3) and abdominal pain, dating from 3 to 6 weeks, with no response to gastro-intestinal dietary, and symptomatic treatments. All patients were treated with symptomatic treatments at the beginning and after complementary examinations and effects, the treatments were completed or changed.

The dogs considered in this study presented ages between 9 and 13 years of age.

A completed blood count test revealed a moderated anaemia (Htc=20-24%) in n=26, and moderate to severe anaemia in the rest of 10 dogs with chronical signs of vomiting, diarrhoea and gastric haemorrhage (19%), a thrombocytosis and leucocytosis at 90000/µl with reference ranges 4000-15000/µl (neutrophilia, eosinophilia, monocytosis and lymphocytosis) in n=30.

A serum biochemical profile revealed hypoalbuminemia at severe to moderate 9-15 g/L in n=15 (reference range 26-35 g/L), an increased valour of alkaline phosphatase 750 U/L (reference range 20-150 U/L) noted in n=19, augmented blood urea nitrogen in n=20 at 50-80 mg/dL (reference range 7-25 mg/dL), and a hypercalcemia in 16 of 36 patients. An augmented valour was remarked for cPL test

(pancreatitis) in liaison with serum bile acid concentration due to hepatic disorders in n=25 in serum cobalamin analysis in n=28 (cPL: 500 µg/L, reference ranges (0-330 µg/L; serum cobalamin: 1200-1400, reference ranges 251-908 ng/L), and high values liver enzyme in n=16 (40-50 UI/L; reference ranges: <40 UI/L), including also higher values of ASAT (57 UI/L, reference ranges:10-50 UI/L) and ALAT (259 UI/L, reference ranges <80 UI/L).

Imaging diagnosis and macroscopic features

Ultrasonographic examination revealed thickening of the gastric wall in pylorus, lesser and greater curvature (hypoechogenic) in 26/36, and the presence of homogenous masses at these levels, measuring 2, 3 and 4 mm, with loss of the normal gastric wall architecture, visible at ultrasound examination in 20/36.

The endoscopic examination revealed the presence of multiple polypoid masses measuring between 8-10 mm diameter, localized in the lesser or greater curvature in n=20. Heavily enlarged folds of gastric mucosa and polypoid mass raised suspicion for malignancy (Figure 1a).

On the pyloric antrum and lesser curvature, multiple diffused and severely oedematous stenosing lesions were observed in n=32 (Figure 1 b and 1d). In the pyloric region it can be noted a haemorrhagic area of a tumour with an irregular surface (Figure 1b).

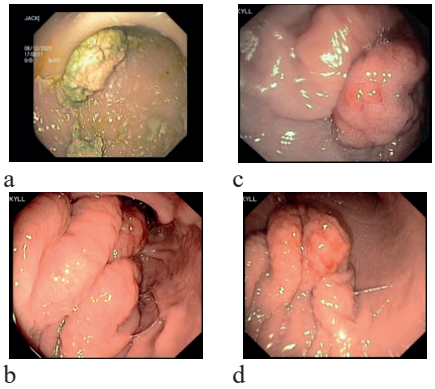


Figure 1. Swiss White Shepherd, 10 years old, polypoid masses (a); Golden Retriever, 13 years old, diffused and oedematous lesions of the pyloric antrum with hypertrophic folds (b); Collie, 8 years old, Irregular and erythematous mass (c); Jack Russel, 12 years old, infiltrating lesion in lesser curvature (d)

At the end of the greater curvature, a highly irregular and erythematous mass focused on several parietal folds was identified (Figure 1c). In the Figure 1d, it was noted eventuated lipped margins around an ulcerative mass. The regional lymph-nodes were involved in n=22.

Macroscopic changes were noticed following necropsy and gastrotomy surgery as oedematous and irregular lesions with severely multifocal haemorrhagic areas visualized at the greater and lesser curvature of the stomach in n=15. The lesions were diffuse and infiltrative, with no marked or unclear ulceration margins, the gastric wall being thickened and indurated (Figure 2 a).

In 9 patients, the structure of the gastric mucosa was granular containing reddened folds as a result of connective tissue hypertrophy, measuring 9-11 cm (Figure 2 b).

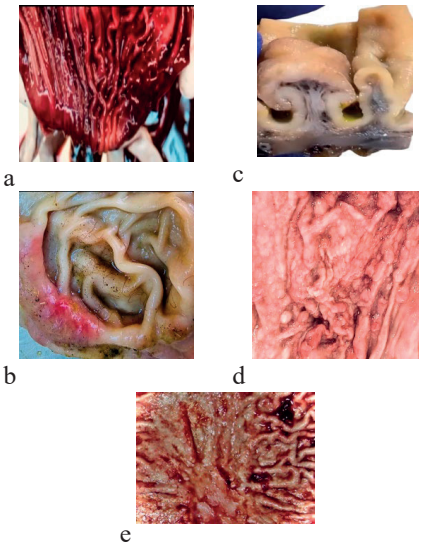


Figure 2. Diffused and increased thickened area, with a prominent ulceration and gastrorrhagia (greater curvature) (a); Localized and high thickening plaque, with reddened folds (greater curvature) (b); Raised polypoid lesion expanded into the lesser curvature (c); Non-ulcerative multiple nodules (d); Ulcerative areas in lesser curvature(e)

The pedunculated masses were observed in a total of 24 cases, measuring more than 10 mm in n=15, and less than 5 mm in n=5 (reference ranges: 2-5 mm), being delimitated by a deep depression from peripheric mucosa. The sessile lesions were presented as nodules being

localized in lesser and greater curvature. Their mucosa was friable and ulcerative (Figure 2 c).
In n=17, multiple giant and exophytic firm nodules adherent to gastric serosa were localized in greater curvature and fundic regions (Figure 2d). The greater and the lesser curvature presented also hypertrophic process (Figure 2d).
In n=25, ulcerative craters have been identified in the stomach wall (lesser curvature) measuring 9 cm. The ulcer margins were elevated, being in contact with gastric folds (Figure 2e). In 18 patients, persistent lesions were identified as non-circumscribed thickened arrows generated by neoplastic cells invasion in the stomach wall, causing erosive craters, ulcerative gastritis, and submucosal fibrosis (Figure 2e).

Cytological examination

In most of the cases (n=28), the cytological examination revealed spherical cells, organized in monolayered adhesive sheets (Figure 3), with an well-defined cytoplasm (Figure 3). Nuclei were round-shaped, being positioned centrally or eccentrically, containing basophilic nucleoli.

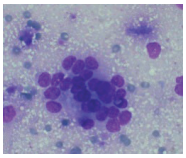


Figure 3. Population of monomorphic epithelial cells (May Grünwald Giemsa staining; x500)

The chromatin was observed as coarse. As malign criteria, the nuclear enlargement, the irregular nuclear membrane, the anizokariosis/ anisonucleosis and the giant nuclei were noted (n=30). The cells were individuals, containing nuclear pleomorphism, and the cytoplasm was well defined. Anisocytosis and anisokaryosis processes were moderate to pronounced, and the N/C proportions were identified as moderate to high (Figure 3).

Histopathological assessment

All samples of stomach, diagnosed with gastric adenocarcinoma diagnosis were classified according to the WHO classification, the results being presented in Table 1.
Neoplastic ducts with various size and dimensions, randomly organized, generating cerebriform formations, specific features in tubular adenocarcinoma, were noted in Figure 4a, in n=9, localized in small and greater curvature. The cells had a polypoid arrangement form, being irregularly distended with atypical and enlarged nuclei. It was noted that sometimes, the cells were as branching tubules with various sizes with mucus and debris (Figure 4a)

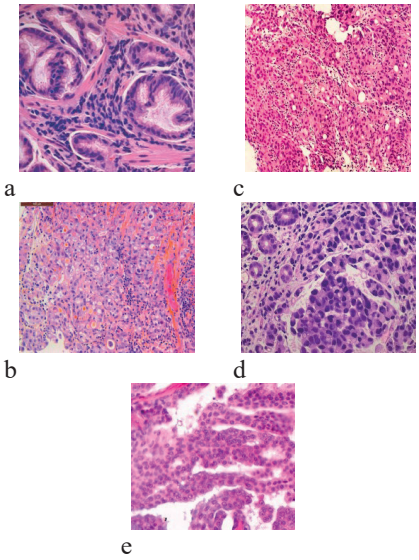


Figure 4. Swiss White Shepherd, 9 years old, Gastric neoplastic ducts with varying size, H.E., x500 (a); Golden Retriever, 12 years old, Signet-ring cell carcinoma, H.E., x300 (b); Border Collie, 8 years old, Undifferentiated adenocarcinoma, H.E., x200 (c); Jack Russel Terrier, 9 years old, Infiltrative mass in pyloric area, H.E., x300 (d); Labrador, 11 years old, Gastric hypertrophy, H.E., x200 (e)

Table 1. Histopathological gastric tumour types

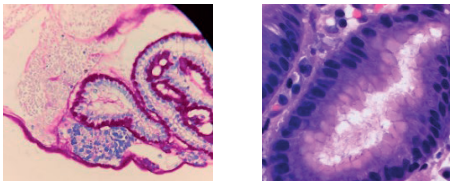
N° cases/36	GA type resulted
14	signet-ring cell adenocarcinoma
9	tubular adenocarcinoma
10	papillary adenocarcinoma
3	undifferentiated adenocarcinoma

A mixture of signet ring adenocarcinoma cells and non-signet ring cells was noted in n=14. It was characterized by a diffuse type and asymmetrical microtrabeculae, associated with significant desmoplasia (Figure 4b).
The mucin-producing cells were identified and the neoplastic cell population showed no indication of glandular or squamous differentiation localized in greater curvature, in n=3 (Figure 4c).

In the Figure 4d, the mass had some tubular glandular differentiation with mucin, but the most of it was composed of undifferentiated tiny cells (poorly differentiated) (Figure 4d).
A high-grade gastric dysplasia, evident hyperplasia and papillary epithelial forms with small fibrous areas, in n=14, suspected to be compatible with papillary adenocarcinoma, located in greater curvature. The cells had a basaloid form, with atypical and enlarged nuclei. The large nucleoli were in mitosis process. In these cases, the regional lymph-nodes were involved (Figure 4e).
In 9 cases diagnosed with signet ring-cell carcinoma, inflammatory cells (neutrophils and eosinophils) were noted in a high quantity, in comparison with tubular and papillary adeno-carcinoma which consisted in lower quantity of inflammatory cells.
Histological analysis of stomach segments, realized in all 36 cases, revealed severe gastritis (ulcerative or non-ulcerative) and gastric cancer, as shown in Table 1.

***Helicobacter pylori* analysis**

ELISA serology test was positive in 20 cases. The Haematoxylin-Eosin coloured smears showed *Helicobacter* spp. as blue-purple colour (Figure 5a) and red (Figure 5b) in 4/36 of cardia samples, in 13/36 of fundus samples, in 6/36 of body samples, and in 4/36 of pylorus samples. It measured between 0.3-0.7µm. *Helicobacter* spp. has been identified in abundance in the mucus that coats the surface gastric epithelium, glandular lamina, and mucosa's surface in the stomach fundus as numerous spiral-shaped organisms (Figure 5).



a b

Figure 5. Positive *Helicobacter pylori* and gastritis (a, b) (Hematoxylin-Eosin, x500)

EGFR analysis

The EGFR test was performed in all 36 patients. Gastric cancer cells expressed EGFR mostly in their membrane and cytoplasm, with brown and yellow particles distributed symmetrically (Figure 6).

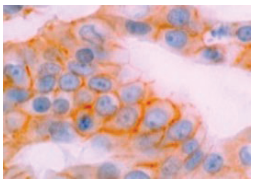


Figure 6. EGFR examination

Table 2. Grades of EGFR expression

Grade of EGFR expression	Breed	Tumour Localization	Histological Diagnosis	EGFR positive/ overexpressed
1	Swiss White Shepherd, French Braque	Fundic and greater curvature	Signet-ring cell adenocarcinoma	EGFR+ in 8
1	Border Collie, Golden Retriever	Pyloric and greater curvature	Poorly differentiated adenocarcinoma	EGFR+in 2
2	Belgian Tervuren, Fox Terrier, French Braque, Golden Retriever	Small and greater curvature	Tubular adenocarcinoma	EGFR+in 10 EGFR severe-expressed in 4
2	Border Collie, Fox Terrier, Golden Retriever,	Greater curvature	Undifferentiated adenocarcinoma	EGFR +severe expressed in 6
3	Labrador, Jack Russel	Pyloric and greater curvature	Papillary adenocarcinoma	EGFR overexpressed n=10

The positive expression of EGFR examination was as graded from 1 to 3 as follows: 1, for the smears with low tumour expression was founded, noted in 10 cases (28%), 2, corresponding to tumour cells staining at positive intensity, in 16 cases (72%) and 3, within the expression of EGFR was overexpressed, in 10 cases (28%) (Table 2).

Therapy and prognostic

The chemo-therapy and surgery protocols were established following clinical evolution and after histopathological analysis results.
In 3 cases, 1 diagnosed with signet-ring cell adenocarcinoma and 2 cases diagnosed with poorly differentiated adenocarcinoma, the chemotherapy treatment (Table 3) was realized in combination with surgical protocols as: partial pilorectomy, for the 1st (<60% of pylori) and partial gastrectomy for another 2 (<50% of stomach removed). No total gastrectomy or gastroduodenal anastomosis were made.
For the rest, the chemotherapy treatment consisted in: intravenous and per-os treatment of: corticotherapy, Toceranib, Chlorambucyl, Cyclophosphamide, Endoxan and Doxorubicine.

Table 3. Treatments and prognostics

Nr. & Breed	Age	Diagnostic & score	Treatment	Prognostic
Swiss White Shepherd (6), French Braque (2)	9-10	Signet-ring cell adenocarcinoma, Grade 1	Vit B12, Corticotherapy, polymixine B, Toceranib	1-4 years
Border Collie (1), Golden Retriever (1)	10-12	Poorly differentiated adenocarcinoma, grade 1	Pylorectomy (1), chlorambucyl	3 months-3 years
Belgian Tervuren (6), Fox Terrier (1), French Braque (1), Golden Retriever (1)	10-11	Tubular adenocarcinoma, grade 2	Chlorambucyl and Cyclophosphamide	16 months-2 years
Border Collie (2), Fox Terrier (2), Golden Retriever (2)	9-12	Undifferentiated adenocarcinoma, grade 2	Endoxan, Cyclophosphamide	10 months
Labrador (2), Jack Russel (8)	10-11	Papillary adenocarcinoma, grade 3	Doxorubicine, Cyclophosphamide	5 weeks to 2-3 months

A chemotherapy prevention with Endoxan, Doxorubicine and Cyclophosphamide was realized in n=10, diagnosed with papillary adenocarcinoma and undifferentiated adenocarcinoma.

In the cases diagnosed with tubular adenocarcinoma and undifferentiated adenocarcinoma, 3 and 4 patients were euthanized 4-5 weeks following the chemotherapy due to resumption of anorexia and regurgitation. The other 7 patients died in the meantime with treatment (2 diagnosed with signet-ring-cell carcinoma and 4 with papillary adenocarcinoma). Median survival time for the patients diagnosed with signet ring cell-adenocarcinoma, with surgical and chemo-therapy treatment was registered between 1-4 years (75%), and one of the other three cases with no responses at treatment, was euthanized after 1 month of therapeutically protocols. For the patients diagnosed with poorly differentiated adenocarcinoma, the median survival was noted between 3 months-3 years, having no metastasis process. The dogs with tubular adenocarcinoma responded to the treatment after 15 days, and the survival time was comprised between 16 months and 2 years, meanwhile survival period for the dogs with tubular adenocarcinoma was

In patients with papillary adenocarcinoma, post-therapy reactions were noted in n=5 as: pulmonary metastasis, and multiple organs dysfunction.

CONCLUSIONS

Signet-ring cell adenocarcinoma, localized in fundic and greater curvature was identified in 6 Swiss White Shepherd dogs and in 2 French Braque (22%), having grade 1 of EGFR expression. In 1 Border Collie and 1 Golden Retriever, the poorly differentiated adenocarcinoma was noted as diagnosis, having grade 1 of EGFR expression. Tubular adenocarcinoma was confirmed in 6 Belgian Tervuren, 1 Fox Terrier, 1 French Braque, 1 Golden Retriever, graded with 2, as EGFR expression. 2 Border Collie, 2 Fox Terrier, and 2 Golden Retriever were diagnosed with Undifferentiated adenocarcinoma, with 2nd grade EGFR expression. 3rd grade of EGFR expression and Papillary adenocarcinoma were identified in 2 Labradors, and 8 Jack Russel.

The survival percentage of signet-ring-cell patients (50%) was significantly distinct from that of patients with poorly differentiated adenocarcinoma (100%), tubular adenocarcinoma (66%), undifferentiated adenocarcinoma (33%), and papillary adenocarcinoma (60%). Poorly differentiated adenocarcinoma and signet-ring-cell patients had a better prognosis than undifferentiated adenocarcinoma. The best prognosis was noted in poorly differentiated adenocarcinoma. In terms of clinicopathological and complementary examinations characteristics, undifferentiated adenocarcinoma patients had a bigger age diversity (9-12). The highest grade of EGFR expression was noted in papillary adenocarcinoma.

The location of tumoral type, tumour size, cell differentiation, and invasion were all correlated with EGFR expression. All complementary examinations and EGFR assessments had an impact in gastric adenocarcinoma tumours progression, promotion and chemotherapy.

Ultrasonography and endoscopy examinations have advantages for diagnosing gastric adenocarcinoma. Endoscopy is far more precise in detecting gastric neoplasia; nevertheless, ultrasounds raise the clinical signs suspicion of gastric neoplasia and may be a less intrusive method of acquiring information before endoscopy (Marolf A., 2015) The subtypes of gastric adenocarcinoma diagnosed after cytopathological and histopathological exams was correlated with necropsy analysis in the meantime when biopsies were done. The results from grade expression of EGFR and severity neoplasia lesions aspects noted during necropsies confirm the theory that the chronicity

neoplastic lesions were related to age, dynamic and progressive process, being a superficial gastritis at the beginning, finishing with an evident gastric atrophy (Rotterdam H., 1981)
Helicobacter pylori infection determined higher grades and intensity of EGFR expression and caused damage gastric epithelial cells.
Canine gastric cancer, similar human stomach cancer, has a very bad prognosis, and current therapy options do not greatly improve survival. The need for new therapies options encourages in-depth research into the role of the complementary examinations as immune-histochemistry (EGFR), in combination with cytology and histopathology (Hugens S., 2017)
Furthermore, there are significant parallels between human and canine gastric cancer in terms of clinical presentation and histological findings, indicating that the dog might serve as a comparison model for human gastric cancer (Hardas A., 2021)
The current study was a complex approach, considering clinical, imaging diagnosis and pathological methods in order to achieve a certain diagnosis and estimate better the prognosis of GA in dogs. Using the WHO principle, this study demonstrated that the most prevalent subtype was papillary carcinoma, tubular adenocarcinoma, and undifferentiated adenocarcinoma. There were no sex variations, and the average age was eleven years of age *Helicobacter* spp. presence was related with elevated chronic inflammation parameters and an expanded chronic inflammatory process, which produced tumour developments (Patnaik A.K., 1980)
Rutine cytology, histopathology, and immune-histochemistry examinations had 78% and 91% accuracy, respectively, in classifying tumour categories in the present cases, being based on biopsy data from surgeries and necropsies.
EGFR is transmembrane tyrosine kinases that increased the accuracy of the diagnostic for gastric neoplasm for the cases considered in this study. The expression of EGFR appeared to be strongly connected, with one or both proteins typically overexpressed in gastric adenocarcinoma cells (Rowinsky, 2004)

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Abbreviations

GA - gastric adenocarcinoma
EGFR - Epidermal Growth Factor Receptor
FFPE - formalin-fixed and paraffin-embedded
FNA - fine needle aspiration
PBS - Phosphate buffered saline
PCR - Polymerase chain reaction
pH - Acid-base production