Veterinary Integrative Sciences 2025; 23(1): e2025020-1-25. DOI: 10.12982/VIS.2025.020

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)

Review article

Protein biomarkers of feline mammary carcinoma

Wajahat Masood [1*], Abdul Wahab Ali[1], Afnaz Shaheen[1], Aeman Zulfiqar[1], Muhammad Saad Ul Haq[1], Syed Ali Raza[1], Ahsan Ashraf[1] and Mehrullah[1]

[1]Faculty of Veterinary and Animal Sciences, PMAS Arid Agriculture University, Rawalpindi 46210, Pakistan

Abstract

Feline mammary carcinoma (FMC) is a prevalent aggressive malignancy in cats, characterized by a poor prognosis and limited treatment options beyond mastectomy. This review explores the significance of various protein biomarkers, including HER2, BCL-2, RON, Ki 67, and COX-2, in diagnosing and treating FMC in cats. These biomarkers have a distinct expression in cancerous breast tissue and are minimally expressed in healthy ones. Early diagnosis and treatment due to the aggressive nature of FMC is important, which often leads to limited therapeutic options and poor responses in later stages. Biomarkers are crucial in improving diagnostic accuracy, guiding therapeutic decisions, and monitoring disease progression in cats with FMC. The diagnosis of these biomarkers is largely based on immunohistochemical and serum analysis. The review highlights the potential of biomarkers to revolutionize diagnostic approaches in veterinary clinics, offering a more targeted and effective strategy for managing feline mammary carcinomas.

Keywords: Biomarkers, Carcinoma, Cats, Diagnosis, Feline

[*]**Corresponding author**: Wajahat Masood, Faculty of Veterinary and Animal Sciences, PMAS Arid Agriculture University, Rawalpindi, 46210, Pakistan. Email Address: wajahatmasood8@gmail.com

Article history; *received manuscript: 12 March 2024,*
revised manuscript: 2 May 2024,
accepted manuscript: 22 May 2024,
published online: 30 May 2024,
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

In developed countries, cats are the most popular companion animals followed by dogs (Murray et al., 2015). The risk of cancer in cats and other domestic animals increases with their prolonged lifespan (Zappulli et al., 2015). Feline mammary carcinoma (FMC) is the third highest occurring cancer in felines, leading to a 17% share in all kinds of tumors in cats. FMC is the leading cause of death in cats, along with a grave prognosis and short survival time; it evades diagnosis at early stages which leads to limited therapeutic options and poor response in later stages (Vail and Macewen, 2000; Zappulli et al., 2005).

Based on molecular differentiation, feline mammary cancers and human breast cancers can be categorized into four different subtypes depending on their malignant character i.e., Luminal A, Luminal B, Her2-positive and triple-negative breast cancer (TNBC) (Soares et al., 2016a; Solinas et al., 2017). Biomarkers improve the efficacy of diagnoses and offer relative therapeutic regimens as compared to traditional tumor therapies (Sarker et al., 2007). Numerous proteinaceous biomarkers have been approved in clinical diagnosis; commonly including human epidermal growth factor (HER2), progesterone and estrogen receptors (ER and PR), cytokeratin, etc. (Füzéry et al., 2013). HER2 protein expression was detected in samples obtained from cats with invasive mammary carcinomas (Muscatello et al., 2019). Soares, 2022. inspected 47 mammary lesions out of which 91.5% lesions were positive for ER. V-domain immunoglobulin suppressor of T cell activation (VISTA) is expressed in aggressive cancers in cats; cats showing luminal A, HER2-positive, or triple-negative tumor subtypes presented higher serum VISTA levels than the healthy population (Gameiro et al., 2021a). Cats with SDF-1 values >2ng/ml had 53 times more chance of developing HER2-positive tumors and higher values (4ng/ml) differentiated HER2-positive and negative tumors (Marques et al., 2017). Syselet al., 2015. conducted a study regarding transcobalamin receptors in malignant tissues, staining positive for TCII, TCII-R, and Ki-67 proteins and having a positive correlation between occurrence of TCII and TCII-R. Another study mentioned the significance of cytotoxic T-lymphocyte-associated proteins (CTLA-4) being positively correlated with feline mammary carcinomas, having higher levels in smaller tumors (Urbano et al., 2020). Cell death proteins PD-1 and PDL-1 levels had a two-fold increase in cats with HER2-positive and triple-negative breast cancer as compared to healthy cats (Nascimento et al., 2020).

IMPORTANCE

FMC tends to be malignant and hormone-independent with aggressive biology, metastasis is observed in 50-90% of cases (Soares et al., 2016). Diagnosis of FMC largely depends on clinical staging, histopathological grading and classification, and molecular assays (Soares et al., 2016). Lack of therapeutics and inefficient chemotherapies (doxorubicin, vincristine) make mastectomy necessary to treat FMC (Michishita et al., 2016). The following review discusses various protein biomarkers prevalent during feline mammary carcinomas which can prove to be a great tool in diagnostic ventures in veterinary clinics.

ETIOLOGY

The etiology of FMC has not been introduced properly but some factors increase the risk namely breed, age, reproductive life, and hormonal exposure (Sorenmo et al., 2013). FMC is more common in middle to old-aged queens which are usually diagnosed between 10 to 12 years of age (Giménez et al., 2010; Morris, 2013). Domestic shorthair cat breeds are highly predisposed to FMC (Hayes Jr et al., 1981; Morris, 2013). Moreover, early-age diagnoses are more common with

Siamese and oriental cat breeds due to germline alterations increasing chances of malignancies (Sorenmo et al., 2013).

The hormonal influence of estrogen and progesterone plays a pivotal role in tumorigenesis of the mammary gland. Cats spayed before one year of age had seven-fold less chance of tumorigenesis than intact cats (Overley et al., 2005; Morris, 2013). Over-exposure of exogenous progestins to control pregnancies has been seen to elevate the risk of benign and malignant FMC (Morris, 2013; Sorenmo et al., 2013). Prolonged exposure to steroids causes rapid proliferation of epithelial cells of the mammary gland which may induce genetic errors side by side, leading to tumorigenesis (Misdorp et al., 1991).

CLINICAL SIGNS

Development of tumors in the mammary gland is normally symptomized as single or numerous nodules or lumps which can either be movable or attached to the connective tissue beneath them (Ameer, 2023). Cats normally tend to groom and lick the area excessively which can lead to ulceration having a strong odor. Generally, signs of illness such as depression and anorexia are seen in increments as the disease progresses (Millanta et al., 2005a; Giménez et al., 2010).

Benign lesions in cats can have varying presentations. Adenomas typically appear as small, solitary, firm nodules, while cysts may feel like beaded or nodular areas in the glands (Hahn et al., 1994). These glands can be edematous, and painful, and may hinder movement, sometimes leading to ulceration. Systemic signs like tachycardia, lethargy, and anorexia may also be present (Görlinger et al., 2002).

Feline malignant mammary tumors typically present as distinct, palpable, and mobile masses. Approximately 25% of cats with these tumors may exhibit ulceration, often accompanied by extensive tumor necrosis (Novosad et al., 2006). Swelling in the pelvic limbs can result from tumor thrombi in the femoral arteries or compromised vascular return from the femoral veins, leading to discomfort, edema, and a decrease in limb temperature. Affected nipples commonly display redness, and swelling, and may discharge tan- or yellow-colored fluid, indicating the severity and complexity of the condition (Giménez et al., 2010).

A thorough physical examination of mammary glands is required to find out the number of glands affected, the size of tumors, location in the gland, and signs of ulceration and inflammation. Palpation of nodules in the mammary gland is recommended (Zappulli et al., 2005). In cases of inflammation, glands will be enlarged and hot and painful on palpation. Teats will also appear swollen and exudative (Ameer, 2023).

DIAGNOSIS

Diagnosis is carried out based on tissue grading and biomarkers found upon blood work.

TISSUE GRADING

CLINICAL STAGING

The clinical staging of the tumor focuses on primary tumor size, lymph node inflammation, and metastasis. The World Health Organization's staging system for feline mammary carcinoma categorizes primary tumors as T1 (< 2cm), T2 (2-3cm), and T3 (> 3cm). Metastasis is defined as M0 (no detection) or M1 (detected). Lymph node involvement is categorized as N0 (no involvement) or N1 (involvement). The stages are defined as T1N0M0 (stage 1), T1-2N0M0 (stage 2), T1-3N0-1M0 (stage 3), and T1-3N0-1M1 (stage 4) (Owen, 1980).

HISTOLOGICAL GRADING

CHOCTEAU ET AL SYSTEM

Histological system formulated by Chocteau et al includes stage 1 with an invasive pathological tumor with a diameter ≤20 mm, negative to unknown nodal status and no lymph node involvement; stage 2 with invasive tumor size > 20mm, negative to unknown nodal status, and no lymph node involvement; stage 3A with invasive tumor size > 20mm, positive nodal status and negative to positive lymph node involvement; stage 3B with invasive tumor size > 20mm, nodal status, and lymph node involvement.

NOTTINGHAM HUMAN GRADING SYSTEM

The NHG system for feline mammary carcinoma, developed by Ellis & Elston, focuses on tubule formation, nuclear pleomorphism, and mitotic count. Each attribute is assigned a score ranging from 1-3, with cumulative scores determining the histological grade of the tumor. Tubule formation scores range from > 75% (score 1) to < 10% (score 3), pleomorphism scores range from normal (score 1) to marked variation (score 3), and mitotic count scores range from 0-8 (score 1) to 9-16 (score 3). The final grade is determined by the total score, with grade 1 scoring 3-5, grade 2 scoring 6-7, and grade 3 scoring 8-9 (Elston and Ellis, 1991).

MILLS ET AL GRADING SYSTEM

Mills et al modified the NHG system to make it more suitable for feline mammary carcinomas. It was based on lymphovascular invasion, nuclear form abnormality, and mitotic count. Lymphovascular invasion may be absent (score 0) or present (score 1), nuclear abnormality may be ≤5% abnormal (score 0) or > 5% abnormal (score 1) and mitotic count may be ≤62 (score 0) or > 62 (score 1). Grade 1 acquires a score of 0, grade 2 acquires a score of 1, and grade 3 acquires a score of 2-3.

IMMUNOHISTOCHEMISTRY SCORING

IHC SCORING BY TAWFIK ET AL

IHC scoring is based on the count of immunopositive cells in the microscopic field. The categories defined by Tawfik et al is defined as + (< 10% immunopositive cells), ++ (10-50% positive cells) and +++ (> 50% immunopositive cells). This classification was conducted on a 400X magnification microscope.

IHC SCORING BY MAGI ET AL

Magi et al devised a 5-score system for immunopositive cells based on the percentage of cells staining positive for immunoreactivity and a 4-score system based on the intensity of staining. The 5-score immunopositive cell percentage system was stated as 0 being no immunoreactivity, 1 being 0-20%, 2 being 21-50%, 3 being 51-70%, and 4 being > 70%. The 4 scores immunopositivity staining system were stated as 0 being no staining, 1 being low-intensity staining, 2 being moderate staining, and 3 being high-intensity staining. The overall score was obtained by multiplying the cell positivity and intensity scores ranging from 0 to 12.

CYTOLOGY

Diagnostic cytology is the examination of a single cell or cell complexes found in the tumor. It is advantageous due to its simple process and quick results (Raskin, 2016). Cytological examinations required fine needle aspiration of the tumor. After the analysis of the slides, the tumor was identified as benign or malignant based on certain attributes stated as anisocytosis, pleomorphism, hypercellularity, macrokaryosis, multinucleation, nuclear molding, high nuclear to cytoplasmic ratio, deformed nuclei, abnormal mitosis, macro/angular nucleoli,

coarse chromatin pattern, anisonucleosis. If the cytological examination yields 3-4 above-mentioned attributes, the tumor is classified as malignant (Simeonov, 2024).

BIOMARKERS

Biomarkers provide the prospective for better diagnostic and improved therapeutic regimens leading to decreased toxicity in contrast to conventional cancer therapies. The ideal biomarker is the one that helps to mark the tumorigenesis at initial stages, devise specific therapy, regulate response to drugs, identify potential drug toxicities, monitor disease prognosis and possible recurrence of cancer (Khleif et al., 2010; Dunstan et al., 2011). Cancers are the result of aberrant molecular and genetic alterations which result in the development of protein products (Makawita and Diamandis, 2010). It is the fluctuation in the level of these proteins that leads to alterations in molecular pathways of malignant and healthy cells (Rhea and Molinaro, 2011). The levels of protein products differ in malignant and healthy tissue which form the basis of diagnosis, thus making the proteins an optimal cancer biomarker. Some of these biomarkers are discussed in detail below.

HER-2

Epidermal growth receptor type 2 (HER 2) protein is a product of the HER 2 gene (neuprotooncogene/c-erb-2). It translates as a 185 kDa transmembrane glycoprotein and is classified into the HER group of the tyrosine kinase receptor family (RTK) (Yamamoto et al., 1986). It has an endogenous tyrosine kinase activity and is involved in normal growth of cells. However, the increased expression of messenger RNA and encoded oncoproteins cause the genomic amplification of Her-2 (Millanta et al., 2005b). In feline mammary carcinoma, amplification of Her-2 indicates that it is a cytogenetic alteration that derives mammary tumor carcinogenesis by providing the cells with proliferative abilities and anti-apoptosis signals (Muscatello et al., 2019). HER protein overexpression has been seen in early tumorigenesis of mammary glands, highly prevalent in ductal carcinomas (50-60%), hence HER 2 can be a differential among mammary carcinoma and atypical hyperplasia (Wärnberg et al., 2002). Many studies have discussed the relationship of HER 2 expressions with clinical outcomes of cancer, tumors expressing HER 2 result in poorer prognosis than HER 2 negative tumors (Menard et al., 2001; Masood and Bui, 2002).

In a study, HER 2 protein was overexpressed in 9 out of 28 (32.1%) of the mammary tumors obtained from dogs (Campos et al., 2015). In the case of dogs, HER 2 overexpression has been associated with high tumor grade, malignancy, large size, and p53 expression in carcinogenesis (Bertagnolli et al., 2011). HER 2 overexpression has also been related to poor prognosis (Dutra et al., 2004). Another study has asserted the absence of HER 2 proteins in normal tissues or benign tumors, hence HER 2 can be used as a typical marker for growth, differentiation, and survival of tumor cells (Tsé et al., 2012).

In cats, most carcinomas expressing HER 2 are classified as solid adenocarcinomas or tubulo-papillary type carcinoma which are associated with clinically aggressive presentation (Marques et al., 2016). Another study reported that cats release some quantities of HER 2 in the bloodstream due to which cats with HER 2 positive mammary carcinomas show elevated levels of serum HER 2 (sHER2). The study determined the optimal serum HER 2 value to be 10ng/ml above which the animal will be considered HER 2 positive (Soares et al., 2016).

BCL-2

B-cell leukemia-2 (BCL-2) was initially identified in B-cell malignancies, associated with integral outer mitochondrial membrane proteins (Tsujimoto and Croce, 1986). BCL-2 has been seen in a variety of normal and cancerous tissues to manage the mitochondrial pathway of apoptosis (Frenzel et al., 2009).

Physiologically, BCL-2 expression is limited to proliferating cell zones such as progenitor and effector cells to inhibit apoptosis and impart longevity in these cells (Madewell et al., 1999).

A human study discussed the prevalence of BCL 2 in mammary tumors. The BCL 2 expression was categorized into high and low. Of the total samples, 52.2% displayed low BCL 2 expression while 47.8% displayed high BCL 2 expression. High BCL 2 expression was concurrent with positive ER and PR status, low tumor grade, and ≤ 50 years of age. High BCL 2 levels were also related to superior disease-free survival and local/systemic recurrence-free survival (Hwang et al., 2021). Similar results were obtained in another study (Al-Alem et al., 2023). In the case of dogs, BCL 2 was associated with histopathological diagnosis while diagnosis was not related to Bax or BCL-XL. Bax expression was increased with clinical staging from T1 to T5 (TNM system). Lastly, BCL 2 and Bax were elevated in malignant tumors as compared to benign ones, but these biomarkers could not determine the survival time (Gurel et al., 2014).

As a pro-survival anti-apoptotic protein, BCL-2 was expected to be related to the aggressive nature of tumors in humans and felines but BCL-2 positivity resulted in better results with luminal FMC in case of disease-free survival. BCL-2 expression yielded 2 fold decrease in cancer-associated health hazards in invasive FMC (Dagher et al., 2019). Such behavior may be due to interaction with other members of the BCL-2 family such as BAG-1, Bad, and Bax (Boise et al., 1993; Oltval et al., 1993; Takayama et al., 1995). BAG-1 inhibits apoptosis (Takayama et al., 1995) while Bad and Bax initiate apoptosis (Yang et al., 1995) along with calcium homeostasis, DNA repair in cancer cells, and autophagy (Juin et al., 2013). BCL-2 expression exhibits a positive prognosis associated with low nuclear grade, ER positivity, and reduced tumor size (Linjawi et al., 2004).

Positive immunohistochemical staining for Bcl-2 was observed in neoplastic cells and some lymphocytes in tumor-associated lymphohistiocytic inflammation. In a study involving 180 FMCs, 72% expressed Bcl-2, with luminal FMCs showing 16% positivity and triple-negative FMCs showing 18% positivity. Notably, Bcl-2 positivity was linked to reduced distant metastasis, squamous differentiation, and tumor-associated inflammation. In luminal and triple-negative FMCs, no significant associations were found with tumor size, nodal stage, grade, or Ki-67 index. However, in basal-like triple-negative FMCs, Bcl-2 positivity was negatively correlated with peritumoral inflammation severity. The study highlighted the favorable prognostic value of Bcl-2 positivity in overall survival and disease-free interval, independent of tumor size and nodal stage (Dagher et al., 2019).

RON

RON (Receptuer d'Origine Nantaise) is a member of the Mesenchymal Epithelial Transition (MET) Receptor of the tyrokinase Family (Danilkovitch-Miagkova, 2003). It is activated by macrophage-stimulated protein (MSP) and is expressed in lower quantities in multiple types of cells for example macrophages, epithelial cells, and hematopoietic cells, involved in mediating growth and inflammatory signaling (Maniscalco et al., 2019; Hunt et al., 2023). However, the overexpression and activation of RON are associated with malignant tumors in multiple tissues in multiple ways. RON signaling overexpression promotes robust tumor cell growth and survival with aiming to facilitate immune evasion while simultaneously blocking the inflammatory pathways (Danilkovitch-Miagkova, 2003; Hunt et al., 2023).

Based on recent discoveries, it is suggested that certain cancer cells might increase the expression of MSP as an extra strategy to evade immune responses against tumors. Additionally, the research indicates that RON plays a crucial role in immunosuppression, even in cases where tumors do not exhibit high levels of MSP. This could be attributed to the activation of circulating MSP by serine proteases derived from macrophages or tumors, highlighting a complex interplay between cancer cells and the immune system in evading antitumor responses (Eyob et al.,

2013). RON constitutes an independent prognostic factor for metastasis and death among breast carcinoma patients (Welm et al., 2007). One study hinted at the fact that MSP and RON were upregulated in BRCA1 deficient tumors than BRCA1 stimulated tumors, suggesting the RON-MSP signaling to be genetically related to unstable, highly genomic tumors (Millar et al., 2020). An immunohistochemistry study revealed that RON expression was highly concurrent with poor relapse-free survival (RFS) and overall survival (OS). Similarly, the analysis of primary and metastatic samples revealed the relation of RON with metastatic spread of tumor. RON had no association with any tumor subtype, nodal status, or tumor stage (Hunt et al., 2020).

It is reported that RON is well expressed in feline mammary tumors – in an experiment using western blot analysis, the mature form of feline RON was identified to be 135 kDa, while the short form of RON was detected in FMCs to be 55 kDa. Hence, it has the potential to predict the outcome of tumorous growth (Maniscalco et al., 2019). Immunohistochemical analysis revealed that 68% of FMCs expressed the RON receptor, while 58% expressed its specific ligand MSP. Co-expression of both proteins was observed in 52% of cases. Notably, expression of the short form of RON (sf-RON) was linked to poorly differentiated tumors and shorter disease-free and overall survival periods. Specifically, sf-RON positivity was associated with a 2.2-fold increased risk of shorter disease-free intervals and overall survival (Maniscalco et al., 2019).

KI 67

Ki 67 was first identified in Hodgkin's lymphoma L428 cells (Gerdes et al., 1983). Healthy mammary glands may express small amounts of Ki 67, exclusively in ER-negative cells. A correlation has been drawn between breast density, the presence of precancerous lesions, and the expression of Ki 67 (Harvey et al., 2008; Zhou et al., 2009). Ki 67 is a protein found in vertebrates that is normally required by the body during the cell cycle except for the G0 phase; for the normal distribution of cellular heterochromatin antigens and the formation of perichromosomal layer (PCL) which is a ribonucleoprotein sheath coating the condensed chromosomes (Sun and Kaufman, 2018). In the case of ductal carcinomas, high levels of Ki 67 have been related to high-grade, necrosis, and microinvasion of other cells (Okumura et al., 2008). Ki 67 also differentiates between luminal A and B subtypes, where luminal tumors expressing at least 14% Ki 67 levels were categorized as luminal B along poor prognosis and death while Ki 67 levels less than 14% were categorized as luminal A (Cheang et al., 2009). Expression of Ki 67 was associated with poor prognosis, lower overall survival, and disease-free survival (de Azambuja et al., 2007).

A study confirms that canine mammary tumors (CMTs) with higher histological grades exhibit increased proliferative activity, as indicated by elevated Ki67 expression. Additionally, a positive correlation was observed between high Ki67 levels and p53 expression, while ER positivity showed an inverse relationship with Ki67 expression. Notably, histological grade, malignancy group classification, and Ki67 expression were found to be significantly linked to overall survival (OS) in CMT patients, although they did not show a significant association with tumor-specific survival (TSS). These results emphasize the prognostic value of Ki67 in CMTs, with higher proliferation indicating more aggressive tumor behavior and poorer outcomes, particularly in ER-negative cases (Brunetti et al., 2021)

Immunohistochemical analysis of Ki-67 expression in feline mammary carcinomas demonstrated moderate to strong nuclear staining in tumor cells, particularly in distant metastases. A significant positive connection was discovered between the Ki-67 index of original tumors and both regional and distant metastases. Interestingly, the Ki-67 index was substantially lower in primary tumors than in distant metastases. Further investigation revealed that a 14% Ki-67 cutoff value may effectively discriminate between highly malignant and low-malignant prospective mammary carcinomas in cats. Primary mammary carcinomas with a

Ki-67 index of ≥14% were related to higher tumor size, poor differentiation (grade III), necrotic regions, negative estrogen receptor (ER) status, and low feline HER2 (fHER2) expression (Soares et al., 2016). As Ki 67 is expressed in cells undergoing mitosis, it is a reliable biomarker for cell proliferation and it is associated with the worst prognosis in feline mammary carcinoma (Sun and Kaufman, 2018).

COX-2

Cyclooxygenase or COX is an enzyme of the myeloperoxidase family. This catalyzes the conversion of arachidonic acid to prostanoids which in turn consist of bioactive proteins such as prostacyclins, thromboxane, and prostaglandins (Chandrasekharan and Simmons, 2004). Physiologically, COX 2 doesn't originate in normal mammary tissue but it is found in premalignant, invasive, and metastatic lesions (Dubios et al., 1998). The involvement of COX-2 as an oncogenesis is complex and least understood in animals but the over-expression of COX-2 can cause the cell phenotype to change from benign to malignant (Greenhough et al., 2009; Rizzo, 2011). COX-2 higher expressions can lead to higher histological grade of tumor malignancy, shortening of overall survival, and worse prognosis (Millanta et al., 2006; Gregório et al., 2017). A meta-analysis on breast cancer found that COX 2 expression was positively related to lymph node metastasis and larger tumor size, the expression was not related to molecular subtypes of BC but it was dependent on vascular invasion of BC. COX 2 overexpression was highly coherent with poor OS and DFS (Xu et al., 2017).

In the case of dogs, COX 2 is expressed in mammary glands in case of inflammation or tumorigenesis. Lymph node metastasis has been evident in cases of high COX 2 expression in CMT (Araújo et al., 2016). Malignant tumors had higher expression of COX 2 than benign ones and expression increased with the increasing grade of tumor (Queiroga et al., 2010). Another study suggested that COX 2 had a role in lymphangiogenic pathways which stimulated angiogenesis in inflammatory mammary cancer in dogs (Clemente et al., 2013).

In the case of cats, IHC studies have yielded 96% positivity in feline mammary tumors. COX 2 expression was related to ER positive and PR negative status of tumors along direct correlation with VEGF expression. COX 2 was associated with a poor prognosis of FMC (Millanta et al., 2006). In terms of COX-2 immunoreactivity, 30% of the cases exhibited low immunoreactivity, while 70% showed high immunoreactivity. High COX-2 expression was significantly linked to various characteristics, including a high mitotic index ($p=0.031$), high histological grade of malignancy ($p\leq0.001$), presence of lymph node metastasis ($p\leq0.001$), vascular invasion ($p=0.002$), disease recurrence ($p=0.019$), and the development of distant metastasis. These findings underscore the association between elevated COX-2 expression and aggressive features in mammary carcinomas, indicating its potential role as a prognostic marker for disease progression and metastatic behavior (Guimarães et al., 2024)

LEPTIN

Leptin is a hormone that is secreted through the adipocytes and crosses the blood-brain barriers to the hypothalamus where it decreases the intake of food and increases metabolism (Havel, 2000; Masood, 2024). There are two isoforms of leptin receptors, one is long (Ob-Rb) and is expressed in the hypothalamus while the short isoforms (Ob-Ra, Ob-Rc, Ob-Rd, Ob-Rf) are expressed in peripheral tissue (Margetic et al., 2002). The function of leptin in tumorigenesis is carried out by attachment to transmembrane receptor ObR (class 1 cytokine receptor family). The binding of leptin to ObR activates certain intracellular pathways such as Akt, JAK2, and MAPK pathways which take part in cell division, differentiation, migration, survival, and proliferation to other tissues. Furthermore, the clinical data and studies also suggest the mediating role of leptin in tumorigenesis and metastasis (Ando et al., 2019). Elevated leptin levels have been associated with

aggressive behavior and poor prognosis of breast cancer (Guo et al., 2012). Leptin levels are fairly increased in cancer epithelium as compared to healthy non-cancerous mammary epithelium (Garofalo et al., 2006).

A study conducted on induced mammary cancer in mice models revealed that leptin expression was significantly higher in mammary tumor tissue as compared to the healthy one. Interestingly, levels of ObR receptors were reduced in mammary tumors. Serum levels of leptin had no significant difference in tumorous or healthy mice (Yilmaz et al., 2022). An IHC study on leptin expression has deduced that leptin expression was more prevalent in invasive ductal carcinoma; whereas in lymphovascular or nodal tumors, leptin expression was negative. Histological grade had an inverse relation with leptin expression with grade 1 having the highest expression. Many tumors expressing leptin were found to be ER and PR-positive. Leptin expression was correlated with luminal A or B subtype of BC while leptin was absent in triple-negative BC (Rasha et al., 2021).

The high level of serum leptin and its over-expression in cats is correlated with tumor size, hormone receptor, and high tumor grade (Jardé et al., 2008). Cats with FMC had significantly low levels of free leptin in the serum in contrast to healthy ones. In terms of tumor subtypes, HER 2 and luminal B subtypes had lower levels of leptin and ObR while luminal A and TNBC had overexpression of these parameters. Serum ObR levels were inversely related to ObR IHC scores, cats with mammary tumors displayed low ObR IHC scores and high serum ObR levels. The cut-off value determined for serum ObR was 16.89ng/ml (Gameiro et al., 2021).

SDF-1

Stromal cell-derived factor-1 is expressed in stromal cells (endothelial cells and fibroblasts) as the name indicates. It attaches to a transmembrane factor called CXCR4 expressed in a variety of tissues and cells. These receptors have been identified on cancer cells too namely brain, lung, prostate, colorectal, and many more but it is to be noted that the prevalence of CXCR4 is nearly nil in normal breast tissues but relatively high in breast cancer cells (Teicher and Fricker, 2010; Zhou et al., 2019). Attachment of SDF-1 to CXCR4, called the CXCR12/CXCR4 axis, results in numerous physiological and pathological mechanisms including apoptosis, mitogenic activity, hematopoiesis, cancer cell growth, and neovascularization (Dewan et al., 2006; Zeng at al., 2017).

CXCL12/CXCR4 axis may be responsible for HER2 activation which increases metastasis in breast cancers (Zhou et al., 2019). Tumor cells expressing CXCR4 promote metastatic spread to organs showing overexpression of CXCL12. Other than cell proliferation, survival, and chemotaxis, the CXCR12/CXCR4 axis also participates in tumor neoangiogenesis by recruiting endothelial progenitor cells which induce expression of VEGF. The combined action of SDF-1 and VEGF leads to the formation of a blood vessel network for the tumor (Teicher and Fricker, 2010; Gelmini et al., 2008). It has been reported that overexpression of CXCR4 in breast cancer cells is positively correlated with distant metastasis and decreased survival in humans, hence modifying the migratory activity of tumor cells towards organs producing SDF-1 (Liang et al., 2005; Zhang et al., 2014). Wang et al., (2022) found in their study that the presence of SDF-1 in serum samples can identify chemosensitive mammary cancers in the case of TNBC.

A study examining the prognostic value of SDF-1 in breast cancer found that patients with serum SDF-1 concentrations above 42pg/ml had a 5.13 times lower chance of disease relapse (Zarychta et al., 2021). In stage I and II BC, CXCR4 levels were significantly elevated compared to healthy individuals. Notably, in stage III and IV BC, plasma concentrations of CXCL12 were markedly higher than in stage I, hinting at a possible role for CXCL12 in identifying early-stage breast cancer (Dąbrowska et al., 2020).

Cats with CXCR4-positive carcinoma exhibit significantly lower serum CXCL12 levels than cats with CXCR4-negative mammary carcinomas (Zielińska and Katanaev, 2020). Significant differences in overall and disease-free survival

curves between the cats with CXCL12 positive and CXCL12 negative tumors are found. Conclusively, CXCL12-negative PT is associated with unfavorable prognosis in cats with HER2-overexpressing tumors (Marques et al., 2018). Cats with mammary carcinoma exhibited higher serum SDF-1 levels (mean value:8.76 ng/ml; range of values: 0.45-36.72 ng/ml) than the healthy cats which showed (mean = 1.28 ng/ml; range of values: 0.38-2.69) ng/ml), with a significant p-value of 0.035 (Marques et al., 2017).

TOPOISOMERASE IIß BINDING PROTEIN 1 (TOPBP1)

TopBP1 has been identified as an interacting partner of topoisomerase IIβ in a yeast two-hybrid screen (Yamane et al., 1997). The eukaryotic type-2A enzyme, topoisomerase II, is the second most abundant chromatin protein after histones. TopBP1 assists in DNA replication initiation and repair, checkpoint signaling, and impact transcription control (Sokka et al., 2010; Wardlaw et al., 2014). TOPBP1 is composed of eight BRCT domains (BRCT1-BRCT8) along with an ATR activating domain situated between BRCT6 and BRCT7 (Blackford et al., 2015). The BRCT1/2 domains of TOPBP1 interact with phosphorylated RAD9, Treslin, and MDC1 to trigger checkpoint signaling, initiate DNA replication, and uphold genomic stability (Delacroix et al., 2007; Leimbacher et al., 2019). Furthermore, the BRCT4/5 motifs bind to phosphorylated 53BP1 or BLM, controlling DNA damage response or thwarting chromosomal abnormalities (Cescutti et al., 2010; Blackford et al., 2015). Additionally, the interaction of the BRCT7/8 domains of TOPBP1 with PLK facilitates the phosphorylation of RAD51, promoting the formation of RAD51 IRIF at sites of double-strand breaks (DSB) (Moudry et al., 2016). Moreover, TOPBP1 modulates the activity of p53 by inhibiting its promoter-binding function through an interaction between the BRCT7/8 domains of TOPBP1 and the DNA-binding domain of p53. This interaction leads to abnormal cell proliferation, increased resistance to chemotherapy, and altered p53 functionality (Liu et al., 2009).

Conducting immunohistochemistry on mammary tissues of human samples unveiled TopBP1 expression in normal breast duct, lobular lining epithelium, and myoepithelium. The staining emphasized nuclear localization, occasionally displaying cytoplasmic staining in isolated cells. Of note, there were no significant distinctions observed in staining characteristics or intensity when comparing normal breast tissue to 'tumor adjacent' morphologically normal breast tissue in most cancer cases. These results support the notion that TopBP1 predominantly resides in the nucleus of regular breast tissue, in line with its involvement in DNA replication and repair functions. Despite its role as a DNA replication factor, the proportions of positively stained cells across all samples indicate that TopBP1 may not function as a proliferation marker. Furthermore, no clear associations were found between the intensity or distribution of TopBP1 expression in carcinomas based on factors such as size, estrogen receptor status, grade, or stage (Going et al., 2007).

In a study, TopBP1 was positive in 122 out of 123 samples taken from cats. The staining was chiefly nuclear with additional occurrence in cytoplasm in some tumors. There was a positive correlation between cells stained with TopBP1 and histological grade. Furthermore, it expresses a higher percentage of cell staining in more malignant neoplasms compared to lower-grade neoplasms and non-neoplastic lesions. Many neoplasms which over-express p53 or are ERα negative, show TopBP1 immuno-reactivity (Morris et al., 2008). Similar results were obtained in the case of dogs (Morris et al., 2009).

CYTOKERATINS (CK5/6)

Cytokeratins 5 and 6 belong to basic type 2 cytokeratins having distinct functions; CK 5 binds to CK 14 to produce heterodimers while CK 6 binds to CK 16 (Moll et al., 2008). These cytokeratins act as intermediate filaments, found in epithelial cells. They are considered an important factor in skeletal function and

maintenance of cell structure (Pastuszak et al., 2015). CK 5 and 6 are produced in some cancerous tissues which act as tumor biomarkers, assisting in verifying the prognosis of cancer. Additionally, it has been suggested that CK5/6 immunostaining may be useful in predicting outcomes for urothelial carcinoma (Mai et al., 2016; Hashmi et al., 2018), triple-negative breast cancer (Maeda et al., 2016; Abdelrahman et al., 2017), and other cancers (Chen et al., 2011; Vasca et al., 2014; Ma et al., 2015). In a study, CK5 was the main staining in mesothelioma, basal cell carcinoma of the skin, urothelial carcinoma, thymoma, and salivary gland tumors, but CK6 was prevalent in adenocarcinomas (Völkel et al., 2022). Several studies have established a link between a poorer prognosis and/or undesirable tumor characteristics in malignancies exhibiting a novel expression of a cytokeratin that is not generally observed in the initial normal cell type (Kabir et al., 2014; Safadi et al., 2019; Rao et al., 2020). The changed expression pattern of intermediate filaments appears to be a typical hallmark of cancer cell dedifferentiation as cancer progresses, implying a link to negative tumor characteristics (Dey et al., 2014).

In a tumor analysis study, it was discovered that 21.1% were positive for CK5 and 22.8% for CK6. Of the 2,920 tumors that tested positive for CK5 and/or CK6, 66% showed staining for both, 16% for CK5, and 18% for CK6. The presence of CK5 and CK6 was associated with high grade, as well as the absence of estrogen and progesterone receptors, and "triple negativity" in breast cancer ($p<0.0001$ each). However, the expression of CK5 and CK6 did not show a correlation with the overall survival of breast cancer patients (Völkel et al., 2022). In contrast to the previously mentioned study, almost 80% of TNBC are positive for the basal character, expressing CK5/6, which is associated with poor prognosis and early recurrence (Goldhirsch et al., 2013). According to the research conducted by Ivković et al., among 117 tumor samples examined, 22% displayed immunohistochemical positivity for CK5/6. This positivity was directly linked to the triple-negative phenotype and a higher histological grade (Ivković et al., 2012). In a cancer study, 17.5% were CK5/6 positive, significantly lower than the benign group with 100% positivity ($P<0.001$). A significant relationship in staining intensity was noted between cancer and benign groups ($P<0.001$). Staining intensity correlated significantly with tumor grade, increasing with higher grades. No significant associations were found with other factors such as invasion or lymph node involvement (Naserabad et al., 2023). The tumor's high biologic aggressiveness, association with tissue necrosis, large tumors, and absence of identified molecular-targeted therapy result in the shortest survival time and disease-free interval (Soares et al., 2016a)

Certain studies conducted on feline mammary cancers have been positive for CK5/6. (Peñafiel-Verdu et al., 2012) performed immunohistochemical analysis on 139 carcinomas obtained from feline mammary glands where 38.1% were negative, 27.3% had mild expression, 23.1% had moderate expression and 11.5% had strong expression. Moreover, grade 1 carcinomas had lower expression of CK 5/6 than grade 2 and 3 carcinomas. Another study asserted the nature of CK 5/6 being an indicator of aggressive neoplasia in cats (Soares et al., 2022). Prevalence of CK 5/6 in feline tumors has been associated with shorter survival times (368.9 days) than normal tumors (725 days) (Soares et al., 2016). The presence of CK5/6 will be deemed positive if the tumor tissue displays 1% positive staining in the cytoplasm or cell membrane (Brunetti et al., 2013).

OSTEOPONTIN

Osteopontin is a cytokine-like phosphoglycoprotein, rich in sialic acid and devoid of collagen. Found in extracellular matrix (Rittling and Chambers, 2004; Rangaswami et al., 2006). It is involved in many physiological mechanisms such as wound and fracture management, immune response, angiogenesis, and tissue remodeling; it can also lead to necrosis of heart tissues, atherosclerosis, and autoimmune disorders (Coombes et al., 2016; Ding et al., 2016; Shimodaira et al., 2018). OPN is expressed by a wide spectrum of immune cells, including

lymphocytes, macrophages, eosinophils, natural killer cells, microglia, and dendritic cells (Moorman et al., 2020).

Integrins assist osteopontin in the activation of the PI3K pathway that imparts cytoprotection hence promoting cancer cell survival; moreover, osteopontin-induced stat-3 signaling prevents staurosporine-dependent apoptosis in breast cancer cells via cyclin D1 and BCL-2 expression (Behera et al., 2010). In some contrasting studies, OPN is upregulated in tumor microenvironment and peripheral circulation. OPN expression is directly proportional to poor prognosis in breast cancer patients (Bramwell et al., 2006). OPN promotes tumor growth, tumor cell invasion, metastasis, EMT, drug resistance, stemness, angiogenesis, and immune suppression through cell surface receptors such as integrins and CD44 (Kariya and Kariya, 2022). These receptors allow binding of OPN which activates the process of carcinogenesis. Integrins allow OPN to attach to the extracellular matrix and convert cellular signals while CD44 is involved in invasion, migration, metastasis, and angiogenesis (Senbanjo and Chellaiah, 2017).

Higher OPN expression was detected in a study on primary mammary gland tumor (MGT) samples from animals with bone metastasis compared to those without bone involvement. In MGT samples, patients with higher OPN expression had a significantly lower survival rate. Immunohistochemical analysis revealed diffuse cytoplasmic OPN staining in 36.6% of MGT samples, and higher OPN expression was detected in MGTs with bone metastasis compared to non-metastatic tumors. Interestingly, patient age showed a moderate positive correlation with OPN expression, and age also had a strong negative correlation with disease-free interval and overall survival, suggesting the association between OPN expression and patient survival was independent of clinicopathological features (Grisoni et al., 2023).

In the case of FMC, osteopontin had aggressive expression in malignant tumors (50.73±9.18) in contrast to benign tissues (5.80±2.16), this expression is a marker of poor prognosis (Ozmen et al., 2015).

CTLA-4

Cytotoxic T lymphocyte antigen 4 aka CTLA 4 or CD125 is found to be an antagonist that inhibits immunity against tumor by downregulating the immune response imparted by T cells (Chen, 2004). CTLA 4 resembles CD28 and shares two ligands with it i.e., CD80 (B7-1) and CD86 (B7-2). Response of immune cells, cytokine production, and progression of the cell cycle are inhibited by the interaction of CTLA 4, B7 ligands, and CD28; hence allowing an environment for tumor growth (Pogorelyy et al., 2018). A study has found that CTLA 4 expression along PD-L1 describes aggressive behavior of tumors with a grave diagnosis (Topalian et al., 2015).

A study conducted on high-grade budding tumors in women revealed that lymphocytes stained with CTLA 4 were more prevalent in the high-bud cohort of the study rather than the low-bud cohort with a gradual increase in staining along buds (Savli et al., 2023). Another study found a correspondence of high CTLA 4 expressions in the tumor and low expressions in the interstitial area around the tumor to be a sign of good prognosis, suggesting EMT to be an inhibitor of antitumor immune response (Yu et al., 2015). A canine study asserted that CTLA 4 IHC scores were higher in metastatic canine mammary tumor tissues than in benign ones, moreover, it provides insight into cancers initiating metastasis. The scores were also negatively correlated with survival time (Ariyarathna et al., 2020).

The cats suffering from mammary carcinoma show that there are increased serum CTLA-4 levels concerning the small size of the tumor, absence of tumor necrosis, non-basal status, and also increased in cats with HER-2 positive status (Hu et al., 2017; Liu et al., 2017). Serum CTLA-4 levels were significantly elevated in cats with mammary carcinoma compared to healthy controls. Specifically, 43% of cats with mammary carcinoma had detectable serum CTLA-4, with a median level of 459.4 pg/mL, while CTLA-4 was undetectable in all healthy cats to such

magnitude (31.3 pg/mL). Further analysis revealed that higher serum CTLA-4 levels were associated with smaller tumor size, absence of tumor necrosis, non-basal tumor status, and HER-2-positive tumors. These findings suggest that CTLA-4 may play an important role in the clinical features and progression of feline mammary carcinoma (Urbano et al., 2020).

VISTA

V-domain Ig suppressor of T cell activation (VISTA, VISR, PD-1H, and c100rf54) was identified in 2011 on immune cells and tumor-infiltrating lymphocytes. VISTA acts as an immune checkpoint receptor suppressing activation and multiplication of T cells and cytokines (Wang et al.,2011). VISTA has a distinct dual function of a ligand by binding the co-inhibitory receptor P-selectin glycoprotein ligand-1 in acidic conditions and a receptor by binding the ligand V-set and immunoglobulin domain-containing protein 3 (Johnston et al., 2019; Wang et al., 2019). A high level of VISTA in breast cancer patients is usually related to the greater size of the tumor, high grade, absence of PR and ER receptors, lymph node involvement, and TNBC molecular subtype (Rezouki et al., 2023).

Cao et al. found that VISTA expression was strongly related to a better outcome in individuals with triple-negative breast cancer (TNBC). VISTA was found in 87.8% of immune cells (ICs) and 18.5% of tumor cells (TCs) in a cohort of 254 individuals with untreated TNBC. This expression has been linked to better overall survival (OS) and extended relapse-free survival (RFS) in TNBC patients. Furthermore, VISTA expression was discovered to be favorably linked with specific types of tumor-infiltrating lymphocytes (TILs), specifically CD4+ TILs (Cao et al., 2021). Another study deduced VISTA to be a positive prognosis biomarker as the tumor samples of high allele tumor heterogeneity breast cancer had minimum expression of anti-tumor CD4 and CD8 cells (McDonald et al., 2019).

In a novel study, serum VISTA levels were assessed in queens diagnosed with aggressive mammary carcinoma subtypes, HER2-positive or triple-negative, for the first time (Soares et al., 2016). The results showed significantly elevated serum VISTA expression levels in these subtypes. These findings mirror those reported in human breast cancer studies, indicating a shared role of the VISTA protein across species (Burugu et al., 2017; Zong et al., 2020). Interestingly, cats with the luminal A tumor subtype also exhibited increased serum VISTA levels, which were linked to the metastatic process (Gameiro et al., 2021). Contrastingly in this study, VISTA-positive TILs were significantly higher in grade 2 FMC than in grade 3 which is associated with a good prognosis (Gameiro et al., 2021). There is a positive correlation seen in the level of serum VISTA and some other immune inhibitory checkpoints, such as serum CTLA-4, PD-1, PD-L1, TNF-α and IL-6 (Gao et al., 2017; ElTanbouly et al., 2020). A high concordance rate is found between serum VISTA level and VISTA-positive cancer cells in feline mammary carcinoma which exhibits VISTA protein as a non-invasive diagnostic biomarker (Papadaki et al., 2020).

PROGRAMMED DEATH LIGAND-1 (PD-L1 AND PD-1)

The expression of the PD-1 molecule is on the cell surface of CD4+, CD8+, NK cells, macrophages, and dendritic cells (Bally et al., 2016). The interaction of PD-1 with PD-L1 can downregulate the expression of some antiapoptotic molecules, and proinflammatory cytokines, and suppress T-cell proliferation by inhibiting the T-cell receptor signaling (McDermott and Atkins, 2013; Baumeister et al., 2016). In terms of tumorigenesis, PD-1 along with its ligands, PDL-1 and PDL-2, are stimulants of T cells and cytotoxic activity thus degenerating anti-tumor immune response (Ohaegbulam et al., 2015). PD-1 has dual contradicting activities. On one hand, it maintains immune tolerance by eliminating harmful immune action; on the other hand, it causes the dilation of malignant cells which hinders the immune response (Salmaninejad et al., 2018). Programmed death ligand 1 (PDL-1)

has Pro tumorigenic action which activates the survival mechanism, this action favors subsequent tumor progression (Dong et al., 2018). PD-L1 expression is generally associated with poor prognosis and aggressive clinicopathological features in breast cancer (Qin et al., 2015; Kim et al., 2017; Wang et al., 2017) while in cases of TNBC, PD-L1 expression translates to better survival (Vonderheide et al., 2017; Stovgaard et al., 2018).

Immunohistochemistry study in canines displayed high staining scores of PD-L1 in mammary tumors that developed metastasis, there was a significant decrease in IHC scores of benign tumors as compared to malignant ones. A significant negative correlation was found between IHC scores and shorter survival in malignant carcinomas (Ariyarathna et al., 2020). Sabatier and group associated PD-L1 expression with features of unfavorable prognosis like large-sized carcinoma, high grade, negative ER and PR status, HER2 and Ki67 positive status (Sabatier et al., 2015).

The feline PD-1 nucleotide sequence in cats was reported to show a sequence of amino acids like that of dogs and humans (Igase et al., 2022). A study reported that serum PD-1 and PD-L1 levels were significantly elevated in cats with HER2-positive and triple-negative (TN) normal-like mammary carcinomas (Nascimento et al., 2020). Studies have correlated PD-1 and PD-L1 to be associated with aggressive subtypes of mammary carcinoma with poor prognosis (Solinas et al., 2017; Noske et al., 2019). such study in cats displaying HER2 positive or normal-like carcinoma revealed elevated levels of serum PD-1 and PD-L1 by 2 to 3 folds. furthermore, elevated serum PD-1 levels, PD-1-positive TILs, and PD-1-positive cancer cells were found in 61.5%, 41.7%, and 100% of cats with HER2-positive mammary carcinoma and in 57.1%, 33.3%, and 100% of cats with triple-negative normal-like mammary tumors. In parallel, elevated serum PD-L1 levels, PD-L1-positive TILs, and PD-L1-positive cancer cells were detected in 76.9%, 66.7%, and 100% of cats with HER2-positive tumors and in 85.7%, 20%, and 100% of cats with a triple-negative normal-like tumor subtype (Nascimento et al., 2020). The serum PD-1 and PD-L1 levels are used as noninvasive biomarkers to diagnose HER2-positive and TN carcinoma and there are strong positive correlations detected between serum PD-1, PD-L1, CTLA-4 and TNF- α levels (Nascimento et al., 2020).

CONCLUSIONS

Biomarkers are pivotal in enhancing diagnostic accuracy and tailoring specific therapeutic approaches, offering a more effective alternative to traditional tumor therapies. The document emphasizes the importance of biomarkers like HER-2, BCL-2, and RON in diagnosing and treating FMC, highlighting their roles in tumorigenesis, therapy specificity, drug response regulation, toxicity identification, disease prognosis monitoring, and potential cancer recurrence detection. Looking toward the future, the use of biomarkers presents a promising avenue for advancing diagnostic capabilities in veterinary clinics. These biomarkers hold the potential to revolutionize diagnostic ventures by aiding in early detection, personalized treatment strategies, and improved outcomes for cats with FMC. The research underscores the significance of biomarkers in not only diagnosing FMC but also in guiding therapeutic decisions and monitoring disease progression, offering a glimpse into a more targeted and effective approach to managing feline mammary carcinomas.

AUTHOR CONTRIBUTIONS

All authors are an equal contributor to this article and all the data, research, composition, and referencing are done collectively.

CONFLICT OF INTEREST

There is no conflict of interest in the final version of the article.

REFERENCES

Abdelrahman, A.E., Rashed, H.E., Abdelgawad, M., Abdelhamid, M.I., 2017. Prognostic impact of EGFR and cytokeratin 5/6 immunohistochemical expression in triple-negative breast cancer. Ann. Diagn. Pathol. 28, 43-53.

Al-Alem, U., Rauscher, G.H., Alem, Q.A., Kajdacsy-Balla, A., Mahmoud, A.M., 2023. Prognostic value of SGK1 and Bcl-2 in invasive breast cancer. Cancers. 15(12), 3151.

Ameer, T., 2023. Overview of feline mammary tumors. Biological Times. 2, 15-16.

Andò, S., Gelsomino, L., Panza, S., Giordano, C., Bonofiglio, D., Barone, I., Catalano, S., 2019. Obesity, leptin, and breast cancer: epidemiological evidence and proposed mechanisms. Cancers. 11(1), 62.

Araújo, M.R., Campos, L.C., Damasceno, K.A., Gamba, C.O., Ferreira, E., Cassali, G.D., 2016. HER-2, EGFR, Cox-2 and Ki67 expression in lymph node metastasis of canine mammary carcinomas: Association with clinical-pathological parameters and overall survival. Res. Vet. Sci. 106, 121-130.

Ariyarathna, H., Thomson, N.A., Aberdein, D., Perrott, M.R., Munday, J.S., 2020. Increased programmed death ligand (PD-L1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) expression are associated with metastasis and poor prognosis in malignant canine mammary gland tumors. Vet. Immunol. Immunopathol. 230, 110142.

Bally, A.P., Austin, J.W., Boss, J.M., 2016. Genetic and epigenetic regulation of PD-1 expression. J. Immunol. 196, 2431-2437.

Baumeister, S.H., Freeman, G.J., Dranoff, G., Sharpe, A.H., 2016. Coinhibitory pathways in immunotherapy for cancer. Annu. Rev. Immunol. 34, 539-573.

Behera, R., Kumar, V., Lohite, K., Karnik, S., Kundu, G.C., 2010. Activation of JAK2/STAT3 signaling by osteopontin promotes tumor growth in human breast cancer cells. Carcinogenesis. 31(2), 192-200.

Bertagnolli, A.C., Ferreira, E., Dias, E.J., Cassali, G.D., 2011. Canine mammary mixed tumors: immunohistochemical expressions of EGFR and HER-2. Aust. Vet. J. 89(8), 312-317.

Blackford, A.N., Nieminuszczy, J., Schwab, R.A., Galanty, Y., Jackson, S.P., Niedzwiedz, W., 2015. TopBP1 interacts with BLM to maintain genome stability but is dispensable for preventing BLM degradation. Molecular. Cell. 57(6), 1133–1141.

Boise, L.H., González-García, M., Postema, C.E., Ding, L., Lindsten, T., Turka, L.A., Thompson, C.B., 1993. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell. 74(4), 597-608.

Bramwell, V.H., Doig, G.S., Tuck, A.B., Wilson, S.M., Tonkin, K.S., Tomiak, A., Chambers, A.F., 2006. Serial plasma osteopontin levels have prognostic value in metastatic breast cancer. Clin. Cancer. Res. 12(11), 3337-3343.

Brunetti, B., Asproni, P., Beha, G., Muscatello, L.V., Millanta, F., Poli, A., Sarli, G., 2013. The molecular phenotype in mammary tumours of queens: correlation between primary tumour and lymph node metastasis. J. Comp. Pathol. 148(2-3), 206-213.

Burugu, S., Gao, D., Leung, S., Chia, S.K., Nielsen, T.O., 2017. LAG-3+ tumor infiltrating lymphocytes in breast cancer: clinical correlates and association with PD-1/PD-L1+ tumors. Ann. Oncol. 28(12), 2977-2984.

Campos, L.C., Silva, J.O., Santos, F.S., Araújo, M.R., Lavalle, G.E., Ferreira, E., Cassali, G.D., 2015. Prognostic significance of tissue and serum HER2 and MUC1 in canine mammary cancer. J. Vet. Diagn. Invest. 27(4), 531-535.

Cao, X., Ren, X., Zhou, Y., Mao, F., Lin, Y., Wu, H., Sun, Q., 2021. VISTA expression on immune cells correlates with favorable prognosis in patients with triple-negative breast cancer. Front. Oncol. 10, 583966.

Cescutti, R., Negrini, S., Kohzaki, M., Halazonetis, T.D., 2010. TopBP1 functions with 53BP1 in the G1 DNA damage checkpoint. Embo. J. 29(21), 3723–3732.

Chandrasekharan, N.V., Simmons, D.L., 2004. The cyclooxygenases. Genome. Biol. 5, 1-7.

Chen, L., 2004. Co-inhibitory molecules of the B7–CD28 family in the control of T-cell immunity. Nat. Rev. Immunol. 4(5), 336-347.

Chen, Y., Cui, T., Yang, L., Mireskandari, M., Knoesel, T., Zhang, Q., Petersen, I., 2011. The diagnostic value of cytokeratin 5/6, 14, 17, and 18 expression in human non-small cell lung cancer. Oncology. 80(5-6), 333-340.

Chocteau, F., Boulay, M.M., Besnard, F., Valeau, G., Loussouarn, D., Nguyen, F., 2019. Proposal for a histological staging system of mammary carcinomas in dogs and cats. Part 2: feline mammary carcinomas. Front. Vet. Sci. 6, 387.

Clemente, M., Sánchez-Archidona, A.R., Sardón, D., Díez, L., Martín-Ruiz, A., Caceres, S., Peña, L., 2013. Different role of COX-2 and angiogenesis in canine inflammatory and non-inflammatory mammary cancer. Vet. J. 197(2), 427-432.

Coombes, J.D., Choi, S.S., Swiderska-Syn, M., Manka, P., Reid, D.T., Palma, E., Syn, W.K., 2016. Osteopontin is a proximal effector of leptin-mediated non-alcoholic steatohepatitis (NASH) fibrosis. BBA Mol. Basis Dis. 1862(1), 135-144.

Dąbrowska, E., Przylipiak, A., Zajkowska, M., Piskor, B.M., Sidorkiewicz, I., Szmitkowski, M., Lawicki, S., 2020. Possible diagnostic application of CXCL12 and CXCR4 as tumor markers in breast cancer patients. Anticancer. Res. 40(6), 3221-3229.

Dagher, E., Abadie, J., Loussouarn, D., Fanuel, D., Campone, M., Nguyen, F., 2019. Bcl-2 expression and prognostic significance in feline invasive mammary carcinomas: a retrospective observational study. BMC. Vet. Res. 15, 1-13.

Danilkovitch-Miagkova, A., 2003. Oncogenic signaling pathways activated by RON receptor tyrosine kinase. Curr. Cancer. Drug. Targets. 3(1), 31-40.

Delacroix, S., Wagner, J.M., Kobayashi, M., Yamamoto, K., Karnitz, L.M., 2007. The Rad9-Hus1-Rad1 (9–1-1) clamp activates checkpoint signaling via TopBP1. Genes. Dev. 21(12), 1472–1477.

Dewan, M.Z., Ahmed, S., Iwasaki, Y., Ohba, K., Toi, M., Yamamoto, N., 2006. Stromal cell-derived factor-1 and CXCR4 receptor interaction in tumor growth and metastasis of breast cancer. Biomed. Pharmacother. 60(6), 273-276.

Dey, P., Togra, J., Mitra, S., 2014. Intermediate filament: structure, function, and applications in cytology. Diagn. Cytopathol. 42(7), 628-635.

Ding, Y., Chen, J., Cui, G., Wei, Y., Lu, C., Wang, L., Diao, H., 2016. Pathophysiological role of osteopontin and angiotensin II in atherosclerosis. Biochem. Biophys. Res. Commun. 471(1), 5-9.

Dong, P., Xiong, Y., Yue, J., Hanley, S.J., Watari, H., 2018. Tumor-intrinsic PD-L1 signaling in cancer initiation, development and treatment: beyond immune evasion. Front. Oncol. 8, 386.

Dubois, R.N., Abramson, S.B., Crofford, L., Gupta, R.A., Simon, L.S., Van De Putte, L.B., Lipsky, P.E., 1998. Cyclooxygenase in biology and disease. FASEB J. 12(12), 1063-1073.

Dunstan, R.W., Wharton Jr, K.A., Quigley, C., Lowe, A., 2011. The use of immunohistochemistry for biomarker assessment—can it compete with other technologies? Toxicol. Pathol. 39(6), 988-1002.

Dutra, A.P., Granja, N.V.M., Schmitt, F.C., Cassali, G.D., 2004. c-erbB-2 expression and nuclear pleomorphism in canine mammary tumors. Braz. J. Med. Biol. Res. 37, 1673-1681.

Elston, C.W., Ellis, I.O., 1991. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 19(5), 403-410.

ElTanbouly, M.A., Schaafsma, E., Smits, N.C., Shah, P., Blazar, B.R., Noelle, R.J., Mabaera, R., 2020. VISTA re-programs macrophage biology through the combined regulation of tolerance and anti-inflammatory pathways. Front. Immunol. 11, 580187.

Eyob, H., Ekiz, H.A., Welm, A.L., 2013. RON promotes the metastatic spread of breast carcinomas by subverting antitumor immune responses. Oncoimmunology. 2(9), 25670.

Frenzel, A., Grespi, F., Chmelewskij, W., Villunger, A., 2009. Bcl2 family proteins in carcinogenesis and the treatment of cancer. Apoptosis. 14, 584-596.

Füzéry, A.K., Levin, J., Chan, M.M., Chan, D.W., 2013. Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges. Clin. Proteomics. 10, 1-14.

Gameiro, A., Nascimento, C., Correia, J., Ferreira, F., 2021. VISTA Is a diagnostic biomarker and immunotherapy target of aggressive feline mammary carcinoma subtypes. Cancers. 13(21), 5559.

Gameiro, A., Urbano, A.C., Ferreira, F., 2021. Emerging biomarkers and targeted therapies in feline mammary carcinoma. Vet. Sci. 8(8), 164.

Gameiro, A., Nascimento, C., Urbano, A.C., Correia, J., Ferreira, F., 2021. Serum and tissue expression levels of leptin and leptin receptor are putative markers of specific feline mammary carcinoma subtypes. Front. Vet. Sci. 8, 625147.

Gao, J., Ward, J.F., Pettaway, C.A., Shi, L.Z., Subudhi, S.K., Vence, L.M., Sharma, P., 2017. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. Nat. Med. 23(5), 551-555.

Garofalo, C., Koda, M., Cascio, S., Sulkowska, M., Kanczuga-Koda, L., Golaszewska, J., Surmacz, E., 2006. Increased expression of leptin and the leptin receptor as a marker of breast cancer progression: possible role of obesity-related stimuli. Clin. Cancer Res. 12(5), 1447-1453.

Gelmini, S., Mangoni, M., Serio, M., Romagnani, P., Lazzeri, E., 2008. The critical role of SDF-1/CXCR4 axis in cancer and cancer stem cells metastasis. J. Endocrinol. Investig. 31, 809-819.

Giménez, F., Hecht, S., Craig, L.E., Legendre, A.M., 2010. Early detection, aggressive therapy: optimizing the management of feline mammary masses. J. Feline Med. Surg. 12(3), 214-224.

Going, J.J., Nixon, C., Dornan, E.S., Boner, W., Donaldson, M.M., Morgan, I.M., 2007. Aberrant expression of TopBP1 in breast cancer. Histopathology. 50(4), 418-424.

Goldhirsch, A., Winer, E.P., Coates, A.S., Gelber, R.D., Piccart-Gebhart, M., Thürlimann, B., Wood, W.C., 2013. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann. Oncol. 24(9), 2206-2223.

Gong, Z., Kim, J.E., Leung, C.C., Glover, J.N., Chen, J., 2010. BACH1/FANCJ acts with TopBP1 and participates early in DNA replication checkpoint control. Molecular Cell. 37(3), 438–446.

Görlinger, S., Kooistra, H.S., Van den Broek, A., Okkens, A.C., 2002. Treatment of fibroadenomatous hyperplasia in cats with aglepristone. J. Vet. Intern. Med. 16(6), 710-713.

Greenhough, A., Smartt, H.J., Moore, A.E., Roberts, H.R., Williams, A.C., Paraskeva, C., Kaidi, A., 2009. The COX-2/PGE 2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis. 30(3), 377-386.

Gregório, H., Raposo, T., Queiroga, F.L., Pires, I., Pena, L., Prada, J., 2017. High COX-2 expression in canine mast cell tumours is associated with proliferation, angiogenesis and decreased overall survival. Vet. Comp. Oncol. 15(4), 1382-1392.

Grisoni Sanchez, C., Figueiredo, M.L., de Sartori Camargo, L., Benevenuto, L.G.D., Lacerda, Z.A., Fonseca-Alves, C.E., 2023. Is Osteopontin a good marker for bone metastasis in canine mammary gland tumor and prostate cancer?. Animals. 13(20), 3211.

Guo, S., Liu, M., Wang, G., Torroella-Kouri, M., Gonzalez-Perez, R.R., 2012. Oncogenic role and therapeutic target of leptin signaling in breast cancer and cancer stem cells. Biochim. Biophys. Acta (BBA) - Rev. Cancer. 1825(2), 207-222.

Gurel, A., Sonta, M.C.G.B.H., 2014. Evaluation of Bd-2, Bd-XL and Bax expression and apoptotic index in canine mammary tumours [1]. Kafkas. Univ. Vet. Fak. Derg. 20(4), 513-520.

Hahn, K.A., Bravo, L., Avenell, J.S., 1994. Feline breast carcinoma as a pathologic and therapeutic. In. Vivo. 8, 825-828.

Hashmi, A.A., Hussain, Z.F., Irfan, M., Edhi, M.M., Kanwal, S., Faridi, N., Khan, A., 2018. Cytokeratin 5/6 expression in bladder cancer: association with clinicopathologic parameters and prognosis. BMC. Res. Notes. 11, 1-5.

Havel, P.J., 2000. Role of adipose tissue in body-weight regulation: mechanisms regulating leptin production and energy balance. Proc. Nutr. Soc. 59(3), 359-371.

Hayes Jr, H.M., Milne, K.L., Mandell, C.P., 1981. Epidemiological features of feline mammary carcinoma. Vet. Rec. 108(22), 476-479.

Hu, P., Liu, Q., Deng, G., Zhang, J., Liang, N., Xie, J., Zhang, J., 2017. The prognostic value of cytotoxic T-lymphocyte antigen 4 in cancers: a systematic review and meta-analysis. Sci. Rep.7(1), 42913.

Hunt, B.G., Fox, L.H., Davis, J.C., Jones, A., Lu, Z., Waltz, S.E., 2023. An introduction and overview of RON receptor tyrosine kinase signaling. Genes. 14(2), 517.

Hunt, B.G., Wicker, C.A., Bourn, J.R., Lower, E.E., Takiar, V., Waltz, S.E., 2020. MST1R (RON) expression is a novel prognostic biomarker for metastatic progression in breast cancer patients. Breast. Cancer. Res. Treat. 181, 529-540.

Hwang, K.T., Kim, Y.A., Kim, J., Oh, H.J., Park, J.H., Choi, I.S., Hwang, K.R., 2021. Prognostic influences of BCL1 and BCL2 expression on disease-free survival in breast cancer. Sci. Rep. 11(1), 11942.

Igase, M., Inanaga, S., Tani, K., Nakaichi, M., Sakai, Y., Sakurai, M., Mizuno, T., 2022. Long-term survival of dogs with stage 4 oral malignant melanoma treated with anti-canine PD-1 therapeutic antibody: A follow-up case report. Vet. Comp. Oncol. 20(4), 901-905.

Ivković, K.T., Panjković, M., Nikolić, I., Đilas, I.D., Knežević, U.S., 2012. Expression of cytokeratins 5/6 and cytokeratin 17 in invasive breast carcinoma. Vojnosanit. Pregl. 69(12), 1031-1038.

Jardé, T., Caldefie-Chézet, F., Damez, M., Mishellany, F., Penault-Llorca, F., Guillot, J., Vasson, M.P., 2008. Leptin and leptin receptor involvement in cancer development: a study on human primary breast carcinoma. Oncol. Rep. 19(4), 905-911.

Johnston, R.J., Su, L.J., Pinckney, J., Critton, D., Boyer, E., Krishnakumar, A., Korman, A.J., 2019. VISTA is an acidic pH-selective ligand for PSGL-1. Nature. 574(7779), 565-570.

Juin, P., Geneste, O., Gautier, F., Depil, S., Campone, M., 2013. Decoding and unlocking the BCL-2 dependency of cancer cells. Nat. Rev. Cancer. 13(7), 455-465.

Kabir, N.N., Rönnstrand, L., Kazi, J.U., 2014. Keratin 19 expression correlates with poor prognosis in breast cancer. Mol. Biol. Rep. 41, 7729-7735.

Kariya, Y., Kariya, Y., 2022. Osteopontin in cancer: mechanisms and therapeutic targets. Int. J. Transl. Med. 2(3), 419-447.

Khleif, S.N., Doroshow, J.H., Hait, W.N., 2010. AACR-FDA-NCI Cancer Biomarkers Collaborative consensus report: advancing the use of biomarkers in cancer drug development. Clin. Cancer. Res. 16(13), 3299-3318.

Kim, H.M., Lee, J., Koo, J.S., 2017. Clinicopathological and prognostic significance of programmed death ligand-1 expression in breast cancer: a meta-analysis. BMC Cancer. 17, 1-11.

Leimbacher, P.A., Jones, S.E., Shorrocks, A.K., de Marco Zompit, M., Day, M., Blaauwendraad, J., Bundschuh, D., Bonham, S., Fischer, R., Fink, D., Kessler, B.M., Oliver, A.W., Pearl, L.H., Blackford, A.N., Stucki, M., 2019. MDC1 interacts with TOPBP1 to maintain chromosomal stability during mitosis. Mol. Cell. 74(3), 571-583.

Liang, Z., Yoon, Y., Votaw, J., Goodman, M.M., Williams, L., Shim, H., 2005. Silencing of CXCR4 blocks breast cancer metastasis. Cancer. Res. 65(3), 967-971.

Linjawi, A., Kontogiannea, M., Halwani, F., Edwardes, M., Meterissian, S., 2004. Prognostic significance of p53, bcl-2, and Bax expression in early breast cancer. J. Am. Coll. Surg. 198(1), 83-90.

Liu, K., Bellam, N., Lin, H.Y., Wang, B., Stockard, C.R., Grizzle, W.E., Lin, W.C., 2009. Regulation of p53 by TopBP1: A potential mechanism for p53 inactivation in cancer. Mol. Cell. Biol. 29(10), 2673–2693.

Liu, Q., Hu, P., Deng, G., Zhang, J., Liang, N., Xie, J., Zhang, J., 2017. Soluble cytotoxic T-lymphocyte antigen 4: a favorable predictor in malignant tumors after therapy. OncoTargets Ther. 2147-2154.

Ma, Y., Fan, M., Dai, L., Kang, X., Liu, Y., Sun, Y., Chen, K., 2015. Expression of p63 and CK5/6 in early-stage lung squamous cell carcinoma is not only an early diagnostic indicator but also correlates with a good prognosis. Thorac. Cancer. 6(3), 288-295.

Madewell, B.R., Candour-Edwards, R., Edwards, B.F., Walls, J.E., Griffey, S.M., 1999. Topographic distribution of bcl-2 protein in feline tissues in health and neoplasia. Vet. Pathol. 36(6), 565-573.

Maeda, T., Nakanishi, Y., Hirotani, Y., Fuchinoue, F., Enomoto, K., Sakurai, K., Nemoto, N., 2016. Immunohistochemical co-expression status of cytokeratin 5/6, androgen receptor, and p53 as prognostic factors of adjuvant chemotherapy for triple negative breast cancer. Med. Mol. Morphol. 49, 11-21.

Mai, K.T., Ball, C.G., Belanger, E.C., 2016. Noninvasive papillary basal-like urothelial carcinoma: a subgroup of urothelial carcinomas with immunohistochemical features of basal urothelial cells associated with a high rate of recurrence and progression. Appl. Immunohistochem. Mol. Morphol. 24(8), 575-582.

Magi, G.E., Mariotti, F., Pallotta, L., Di Cerbo, A., Venanzi, F.M., 2022. Immunohistochemical expression of p62 in feline mammary carcinoma and non-neoplastic mammary tissue. Animals. 12(15), 1964.

Makawita, S., Diamandis, E.P., 2010. The bottleneck in the cancer biomarker pipeline and protein quantification through mass spectrometry–based approaches: current strategies for candidate verification. Clin. Chem. 56(2), 212-222.

Maniscalco, L., Guil-Luna, S., Iussich, S., Gattino, F., Trupia, C., Millan, Y., De Maria, R., 2019. Expression of the short form of RON/STK in feline mammary carcinoma. Vet. Pathol. 56(2), 220-229.

Margetic, S., Gazzola, C., Pegg, G.G., Hill, R.A., 2002. Leptin: a review of its peripheral actions and interactions. Int. J. Obes. Relat. Metab. Disord. 26(11), 1407-1433.

Marques, C., Correia, J., Ferreira, F., 2016. HER2-positive feline mammary carcinoma. Aging (Albany NY). 8(8), 1574.

Marques, C.S., Santos, A.R., Gameiro, A., Correia, J., Ferreira, F., 2018. CXCR4 and its ligand CXCL12 display opposite expression profiles in feline mammary metastatic disease, with the exception of HER2-overexpressing tumors. BMC. Cancer. 18, 1-13.

Marques, C.S., Soares, M., Santos, A., Correia, J., Ferreira, F., 2017. Serum SDF-1 levels are a reliable diagnostic marker of feline mammary carcinoma, discriminating HER2-overexpressing tumors from other subtypes. Oncotarget. 8(62), 105775.

Masood, S., Bui, M.M., 2002. Prognostic and predictive value of HER2/neu oncogene in breast cancer. Microsc. Res. Tech. 59(2), 102-108.

Masood, W., 2024. The general and systemic consequences of obesity in cats and dogs. Vet. Integr. Sci. 20(1), 265-290

McDermott, D.F., Atkins, M.B., 2013. PD-1 as a potential target in cancer therapy. Cancer. Med. 2(5), 662-673.

McDonald, K.A., Kawaguchi, T., Qi, Q., Peng, X., Asaoka, M., Young, J., Takabe, K., 2019. Tumor heterogeneity correlates with less immune response and worse survival in breast cancer patients. Ann. Surg. Oncol. 26, 2191-2199.

Ménard, S., Fortis, S., Castiglioni, F., Agresti, R., Balsari, A., 2001. HER2 as a prognostic factor in breast cancer. Oncology. 61(2), 67-72.

Maniscalco, L., Guil-Luna, S., Iussich, S., Gattino, F., Trupia, C., Millan, Y., De Maria, R., 2019. Expression of the short form of RON/STK in feline mammary carcinoma. Vet. Pathol. 56(2), 220-229.

Michishita, M., Ohtsuka, A., Nakahira, R., Tajima, T., Nakagawa, T., Sasaki, N., Takahashi, K., 2016. Anti-tumor effect of bevacizumab on a xenograft model of feline mammary carcinoma. J. Vet. Med. Sci. 78(4), 685-689.

Millanta, F., Asproni, P., Canale, A., Citi, S., Poli, A., 2016. COX-2, mPGES-1 and EP2 receptor immunohistochemical expression in canine and feline malignant mammary tumours. Vet. Comp. Oncol. 14(3), 270-280.

Millanta, F., Calandrella, M., Bari, G., Niccolini, M., Vannozzi, I., Poli, A., 2005a. Comparison of steroid receptor expression in normal, dysplastic, and neoplastic canine and feline mammary tissues. Res. Vet. Sci. 79(3), 225-232.

Millanta, F., Calandrella, M., Citi, S., Della Santa, D., Poli, A., 2005b. Overexpression of HER-2 in feline invasive mammary carcinomas: an immunohistochemical survey and evaluation of its prognostic potential. Vet. Pathol. 42(1), 30-34.

Millanta, F., Citi, S., Della Santa, D., Porciani, M., Poli, A., 2006. COX-2 expression in canine and feline invasive mammary carcinomas: correlation with clinicopathological features and prognostic fmolecular markers. Breast. Cancer. Res. Treat. 98, 115-120.

Millar, R., Kilbey, A., Remak, S.J., Severson, T.M., Dhayade, S., Sandilands, E., Coffelt, S.B., 2020. The MSP-RON axis stimulates cancer cell growth in models of triple negative breast cancer. Mol. Oncol. 14(8), 1868-1880.

Mills, S.W., Musil, K.M., Davies, J.L., Hendrick, S., Duncan, C., Jackson, M.L., Simko, E., 2015. Prognostic value of histologic grading for feline mammary carcinoma:a retrospective survival analysis. Vet. Pathol. 52(2), 238-249.

Misdorp, W., Romijn, A., Hart, A.A., 1991. Feline mammary tumors: a case-control study of hormonal factors. Anticancer. Res. 11(5), 1793-1797.

Moll, R., Divo, M., Langbein, L., 2008. The human keratins: biology and pathology. Histochem. Cell. Biol. 129, 705-733.

Moorman, H.R., Poschel, D., Klement, J.D., Lu, C., Redd, P.S., Liu, K., 2020. Osteopontin: a key regulator of tumor progression and immunomodulation. Cancers. 12(11), 3379.

Morris, J., 2013. Mammary tumours in the cat: size matters, so early intervention saves lives. J. Feline. Med. Surg. 15(5), 391-400.

Morris, J.S., Nixon, C., Bruck, A., Nasir, L., Morgan, I.M., Philbey, A.W., 2008. Immunohistochemical expression of TopBP1 in feline mammary neoplasia in relation to histological grade, Ki67, ERα and p53. Vet. J. 175(2), 218-226.

Morris, J.S., Nixon, C., King, O.J., Morgan, I.M., Philbey, A.W., 2009. Expression of TopBP1 in canine mammary neoplasia in relation to histological type, Ki67, ERα and p53. Vet. J. 179(3), 422-429.

Moudry, P., Watanabe, K., Wolanin, K.M., Bartkova, J., Wassing, I.E., Watanabe, S., Strauss, R., Troelsgaard Pedersen, R., Oestergaard, V.H., Lisby, M., Andújar-Sánchez, M., Maya-Mendoza, A., Esashi, F., Lukas, J., Bartek, J., 2016. TOPBP1 regulates RAD51 phosphorylation and chromatin loading and determines PARP inhibitor sensitivity. J. Cell. Biol. 212(3), 281-288.

Murray, J.K., Gruffydd-Jones, T.J., Roberts, M.A., Browne, W.J., 2015. Assessing changes in the UK pet cat and dog populations: numbers and household ownership. Vet. Rec. 177(10), 259-259.

Muscatello, L.V., Di Oto, E., Sarli, G., Monti, V., Foschini, M.P., Benazzi, C., Brunetti, B., 2019. HER2 amplification status in feline mammary carcinoma: a tissue microarray–fluorescence in situ hydridization–based study. Vet. Pathol. 56(2), 230-238.

Nascimento, C., Urbano, A.C., Gameiro, A., Ferreira, J., Correia, J., Ferreira, F., 2020. Serum PD-1/PD-L1 levels, tumor expression and PD-L1 somatic mutations in HER2-positive and triple negative normal-like feline mammary carcinoma subtypes. Cancers. 12(6), 1386.

Naserabad, S.A.R., Bagheri, S., Kheradmand, P., Latifi, S.M., 2023. Investigating the expression of cytokeratin 5/6 in benign and malignant breast lesions. Immunopathol. Persa. x(x), 39477.

Nascimento, C., Urbano, A.C., Gameiro, A., Ferreira, J., Correia, J., Ferreira, F., 2020. Serum PD-1/PD-L1 levels, tumor expression and PD-L1 somatic mutations in HER2-positive and triple negative normal-like feline mammary carcinoma subtypes. Cancers. 12(6), 1386.

Noske, A., Möbus, V., Weber, K., Schmatloch, S., Weichert, W., Köhne, C.H., Denkert, C., 2019. Relevance of tumour-infiltrating lymphocytes, PD-1 and PD-L1 in patients with high-risk, nodal-metastasised breast cancer of the German Adjuvant Intergroup Node–positive study. Eur. J. Cancer. 114, 76-88.

Novosad, C.A., Bergman, P.J., O'Brien, M.G., McKnight, J.A., Charney, S.C., Selting, K.A., Gieger, T.L., 2006. Retrospective evaluation of adjunctive doxorubicin for the treatment of feline mammary gland adenocarcinoma: 67 cases. J. Am. Anim. Hosp. Assoc. 42(2), 110-120.

Ohaegbulam, K.C., Assal, A., Lazar-Molnar, E., Yao, Y., Zang, X., 2015. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends. Mol. Med. 21(1), 24-33.

Oltval, Z.N., Milliman, C.L., Korsmeyer, S.J., 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programed cell death. Cell. 74(4), 609-619.

Overley, B., Shofer, F.S., Goldschmidt, M.H., Sherer, D., Sorenmo, K.U., 2005. Association between ovarihysterectomy and feline mammary carcinoma. J. Vet. Intern. Med. 19(4), 560-563.

Owen, L.N., 1980. TNM Classification of Tumours in Domestic Animals/edited by LN Owen. WHO, Geneva

Ozmen, O., Haligur, M., Ipek, V., 2015. Immunohistochemical expression of osteopontin in canine and feline tumors. Rev. Med. Vet. 166, 2-10.

Papadaki, M.A., Koutsopoulos, A.V., Tsoulfas, P.G., Lagoudaki, E., Aggouraki, D., Monastirioti, A., Agelaki, S., 2020. Clinical relevance of immune checkpoints on circulating tumor cells in breast cancer. Cancers. 12(2), 376.

Pastuszak, M., Groszewski, K., Pastuszak, M., Dyrla, P., Wojtuń, S., Gil, J., 2015. Cytokeratins in gastroenterology. Systematic review. Gastroenterol. Rev. 10(2), 61-70.

Peñafiel-Verdu, C., Buendia, A.J., Navarro, J.A., Ramirez, G.A., Vilafranca, M., Altimira, J., Sanchez, J., 2012. Reduced expression of E-cadherin and β-catenin and high expression of basal cytokeratins in feline mammary carcinomas with regional metastasis. Vet. Pathol. 49(6), 979-987.

Pogorelyy, M.V., Fedorova, A.D., McLaren, J.E., Ladell, K., Bagaev, D.V., Eliseev, A. V., Shugay, M., 2018. Exploring the pre-immune landscape of antigen-specific T cells. Genome. Med. 10(1), 1-14.

Qin, T., Zeng, Y.D., Qin, G., Xu, F., Lu, J.B., Fang, W.F., Xue, C., Zhan, J.H., Zhang, X.K., Zheng, Q.F., Peng, R.J., Yuan, Z.Y., Zhang, L., Wang, S.S., 2015. High PD-L1 expression was associated with poor prognosis in 870 chinese patients with breast cancer. Oncotarget. 6(32), 33972-33981.

Queiroga, F.L., Pires, I., Lobo, L., Lopes, C.S., 2010. The role of Cox-2 expression in the prognosis of dogs with malignant mammary tumours. Res. Vet. Sci. 88(3), 441-445.

Rangaswami, H., Bulbule, A., Kundu, G.C., 2006. Osteopontin: role in cell signaling and cancer progression. Trends. Cell. Biol. 16(2), 79-87.

Rao, X., Wang, J., Song, H.M., Deng, B., Li, J.G., 2020. KRT15 overexpression predicts poor prognosis in colorectal cancer. Neoplasma. 67(2), 410-414.

Rasha, M.R., Yasmine, F.E., Ismail Amer, M.D., Samar, I., 2021. Immunohistochemical expression of leptin in mammary carcinoma. Med. J. Cairo Univ. 89(1), 285-296.

Raskin, R.E., 2016. General categories of cytologic interpretation. Canine Feline Cytology. 15-25.

Rezouki, I., Zohair, B., Ssi, S.A., Karkouri, M., Razzouki, I., Elkarroumi, M., Badou, A., 2023. High VISTA expression is linked to a potent epithelial-mesenchymal transition and is positively correlated with PD1 in breast cancer. Front. Oncol. 13, 1154631.

Rhea, J.M., Molinaro, R.J., 2011. Cancer biomarkers: surviving the journey from bench to bedside. MLO Med. Lab. Obs. 43(3), 10-12.

Rittling, S.R., Chambers, A.F., 2004. Role of osteopontin in tumour progression. Br. J. Cancer. 90(10), 1877-1881.

Rizzo, M.T., 2011. Cyclooxygenase-2 in oncogenesis. Clin. Chim. Acta. 412(9-10), 671-687.

Sabatier, R., Finetti, P., Mamessier, E., Adelaide, J., Chaffanet, M., Ali, H.R., Bertucci, F., 2015. Prognostic and predictive value of PDL1 expression in breast cancer. Oncotarget. 6(7), 5449.

Safadi, R.A., Abdullah, N.I., Alaaraj, R.F., Bader, D.H., Divakar, D.D., Hamasha, A.A., Sughayer, M.A., 2019. Clinical and histopathologic prognostic implications of the expression of cytokeratins 8, 10, 13, 14, 16, 18 and 19 in oral and oropharyngeal squamous cell carcinoma. Arch. Oral. Biol. 99, 1-8.

Salmaninejad, A., Khoramshahi, V., Azani, A., Soltaninejad, E., Aslani, S., Zamani, M.R., Hosseini, S.M., 2018. PD-1 and cancer: molecular mechanisms and polymorphisms. Immunogenetics. 70, 73-86.

Sarker, D., Pacey, S., Workman, P., 2007. Use of pharmacokinetic/ pharmacodynamic biomarkers to support rational cancer drug development. Future. Med. 399-417.

Savli, T.B., Pasaoglu, H.E., Savli, T.C., Muhammedoglu, A., Tokocin, M., Öztürk, Ç., 2023. Expression of cytotoxic T lymphocyte-associated antigen 4, CD44, and E-cadherin in the microenvironment of breast carcinomas. Rev. Assoc. Med. Bras. 69, e20230371.

Senbanjo, L.T., Chellaiah, M.A., 2017. CD44: a multifunctional cell surface adhesion receptor is a regulator of progression and metastasis of cancer cells. Front. Cell. Dev. Biol. 5, 18.

Shimodaira, T., Matsuda, K., Uchibori, T., Sugano, M., Uehara, T., Honda, T., 2018. Upregulation of osteopontin expression via the interaction of macrophages and fibroblasts under IL-1b stimulation. Cytokine. 110, 63-69.

Simeonov, R., 2024. Correlation between fine-needle aspiration biopsy and routine histopathology in the diagnosis of spontaneous feline mammary gland tumours. Bulg. J. Vet. Med. 27(1), 16-33.

Soares, M., Correia, A.N., Batista, M.R., Correia, J., Ferreira, F., 2022. fHER2, PR, ER, Ki-67 and Cytokeratin 5/6 Expression in Benign Feline Mammary Lesions. Animals. 12(13), 1599.
Soares, M., Madeira, S., Correia, J., Peleteiro, M., Cardoso, F., Ferreira, F., 2016. Molecular based subtyping of feline mammary carcinomas and clinicopathological characterization. The Breast. 27, 44-51.
Soares, M., Ribeiro, R., Najmudin, S., Gameiro, A., Rodrigues, R., Cardoso, F., Ferreira, F., 2016. Serum HER2 levels are increased in cats with mammary carcinomas and predict tissue HER2 status. Oncotarget. 7(14), 17314.
Sokka, M., Parkkinen, S., Pospiech, H., Syväoja, J.E., 2010. Function of TopBP1 in genome stability. Genome Stability Hum. Dis. 50, 119-141.
Solinas, C., Carbognin, L., De Silva, P., Criscitiello, C., Lambertini, M., 2017. Tumor-infiltrating lymphocytes in breast cancer according to tumor subtype: current state of the art. The Breast. 35, 142-150.
Solinas, C., Garaud, S., De Silva, P., Boisson, A., Van den Eynden, G., de Wind, A., Willard-Gallo, K., 2017. Immune checkpoint molecules on tumor-infiltrating lymphocytes and their association with tertiary lymphoid structures in human breast cancer. Front. Immunol. 8, 1412.
Sorenmo, K.U., Worley, D.R., Goldschmidt, M.H., 2013. Tumors of the mammary gland. John Wiley & Sons, Hoboken, New Jersey, pp. 538-556.
Stovgaard, E.S., Nielsen, D., Hogdall, E., Balslev, E., 2018. Triple negative breast cancer - prognostic role of immune-related factors: a systematic review. Acta. Oncol. 57(1), 74-82.
Sun, X., Kaufman, P.D., 2018. Ki-67: more than a proliferation marker. Chromosoma. 127, 175-86.
Sysel, A.M., Valli, V.E., Bauer, J.A., 2015. Immunohistochemical quantification of the cobalamin transport protein, cell surface receptor and Ki-67 in naturally occurring canine and feline malignant tumors and in adjacent normal tissues. Oncotarget. 6(4), 2331.
Takayama, S., Sato, T., Krajewski, S., Kochel, K., Irie, S., Milian, J.A., Reed, J.C., 1995. Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. Cell. 80(2), 279-284.
Tawfik, M.F., Oda, S.S., Khafaga, A.F., 2021. Pathological and immunohistochemical microscopy of natural cases of canine and feline neoplastic mammary lesions. Microsc. Microanal. 27(4), 910-922.
Teicher, B.A., Fricker, S.P., 2010. CXCL12 (SDF-1)/CXCR4 pathway in cancer. Clin. Cancer Res. 16(11), 2927-2931.
Topalian, S.L., Drake, C.G., Pardoll, D.M., 2015. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer. Cell. 27(4), 450-461.
Tsé, C., Gauchez, A.S., Jacot, W., Lamy, P.J., 2012. HER2 shedding and serum HER2 extracellular domain: biology and clinical utility in breast cancer. Cancer Treat. Rev. 38(2), 133-142.
Tsujimoto, Y., Croce, C.M., 1986. Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. Proc. Natl. Acad. Sci. USA. 83(14), 5214-5218.
Urbano, A.C., Nascimento, C., Soares, M., Correia, J., Ferreira, F., 2020. Clinical Relevance of the serum CTLA-4 in cats with mammary carcinoma. Sci. Rep. 10(1), 3822.
Vail, D.M., Macewen, E.G., 2000. Spontaneously occurring tumors of companion animals as models for human cancer. Cancer. Invest. 18(8), 781-792.
Vasca, V., Vasca, E., Freiman, P., Marian, D., Luce, A., Mesolella, M., Duminica, T., 2014. Keratin 5 expression in squamocellular carcinoma of the head and neck. Oncol. Lett. 8(6), 2501-2504.
Völkel, C., De Wispelaere, N., Weidemann, S., Gorbokon, N., Lennartz, M., Luebke, A.M., Menz, A., 2022. Cytokeratin 5 and cytokeratin 6 expressions are

unconnected in normal and cancerous tissues and have separate diagnostic implications. Virchows Arch. 480(2), 433-447.
Vonderheide, R.H., Domchek, S.M., Clark, A.S., 2017. Immunotherapy for breast cancer: what are we missing?. Clin. Cancer. Res. 23(11), 2640-2646.
Wang, C., Zhu, H., Zhou, Y., Mao, F., Lin, Y., Pan, B., Sun, Q., 2017. Prognostic value of PD-L1 in breast cancer: a meta-analysis. Breast. J. 23(4), 436-443.
Wang, J., Wu, G., Manick, B., Hernandez, V., Renelt, M., Erickson, C., Kalabokis, V., 2019. VSIG-3 as a ligand of VISTA inhibits human T-cell function. Immunology. 156(1), 74-85.
Wang, L., Rubinstein, R., Lines, J.L., Wasiuk, A., Ahonen, C., Guo, Y., Noelle, R.J., 2011. VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses. J. Exp. Med. 208(3), 577-592.
Wang, R.X., Ji, P., Gong, Y., Shao, Z.M., Chen, S., 2022. SDF-1 expression and tumor-infiltrating lymphocytes identify clinical subtypes of triple-negative breast cancer with different responses to neoadjuvant chemotherapy and survival. Front. Immunol. 13, 940635.
Wardlaw, C.P., Carr, A.M., Oliver, A.W., 2014. TopBP1: A BRCT-scaffold protein functioning in multiple cellular pathways. DNA repair. 22, 165-174.
Wärnberg, F., Casalini, P., Nordgren, H., Bergkvist, L., Holmberg, L., Ménard, S., 2002. Ductal carcinoma in situ of the breast: a new phenotype classification system and its relation to prognosis. Breast. Cancer. Res. Treat. 73, 215-222.
Welm, A.L., Sneddon, J.B., Taylor, C., Nuyten, D.S., van de Vijver, M.J., Hasegawa, B. H., Bishop, J.M., 2007. The macrophage-stimulating protein pathway promotes metastasis in a mouse model for breast cancer and predicts poor prognosis in humans. Proc. Natl. Acad. Sci. 104(18), 7570-7575.
Xu, F., Li, M., Zhang, C., Cui, J., Liu, J., Li, J., Jiang, H., 2017. Clinicopathological and prognostic significance of COX-2 immunohistochemical expression in breast cancer: a meta-analysis. Oncotarget. 8(4), 6003–6012.
Yamamoto, T., Ikawa, S., Akiyama, T., Semba, K., Nomura, N., Miyajima, N., Toyoshima, K., 1986. Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor. Nature. 319(6050), 230-234.
Yamane, K., Kawabata, M., Tsuruo, T., 1997. A DNA-Topoisomerase-11–Binding Protein with Eight Repeating Regions Similar to DNA-repair Enzymes and to a Cell-Cycle Regulator. Eur. J. Biochem. 250(3), 794-799.
Yang, E., Zha, J., Jockel, J., Boise, L.H., Thompson, C.B., Korsmeyer, S.J., 1995. Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. Cell. 80(2), 285-291.
Yilmaz, E., Thomas, P.B., Tuna, B.G., Cleary, M.P., Dogan, S., 2022. Leptin receptors expression in mammary tumors and mammary fat pad of transgenic mammary cancer mouse model. Exp. Oncol. 44(4), 272-280.
Yu, H., Yang, J., Jiao, S., Li, Y., Zhang, W., Wang, J., 2015. Cytotoxic T lymphocyte antigen 4 expression in human breast cancer: implications for prognosis. Cancer. Immunol. Immunother. 64, 853-860.
Zappulli, V., De Zan, G., Cardazzo, B., Bargelloni, L., Castagnaro, M., 2005. Feline mammary tumours in comparative oncology. J. Dairy. Res. 72(S1), 98-106.
Zappulli, V., Rasotto, R., Caliari, D., Mainenti, M., Peña, L., Goldschmidt, M.H., Kiupel, M., 2015. Prognostic evaluation of feline mammary carcinomas: a review of the literature. Vet. Pathol. 52(1), 46-60.
Zarychta, E., Ruszkowska-Ciastek, B., Bielawski, K., Rhone, P., 2021. Stromal cell-derived factor 1α (SDF-1α) in invasive breast cancer: associations with vasculo-angiogenic factors and prognostic significance. Cancers. 13(8), 1952.
Zeng, Y., Wang, X., Yin, B., Xia, G., Shen, Z., Gu, W., Wu, M., 2017. Role of the stromal cell derived factor-1/CXC chemokine receptor 4 axis in the invasion and metastasis of lung cancer and mechanism. J. Thorac. Dis. 9(12), 4947.
Zielińska, K.A., Katanaev, V.L., 2020. The signaling duo CXCL12 and CXCR4: Chemokine fuel for breast cancer tumorigenesis. Cancers. 12(10), 3071.

Zhang, Z., Ni, C., Chen, W., Wu, P., Wang, Z., Yin, J., Qiu, F., 2014. Expression of CXCR4 and breast cancer prognosis: a systematic review and meta-analysis. BMC cancer. 14, 1-8.

Zhou, W., Guo, S., Liu, M., Burow, M.E., Wang, G., 2019. Targeting CXCL12/CXCR4 axis in tumor immunotherapy. Curr. Med. Chem. 26(17), 3026-3041.

Zong, L., Mo, S., Yu, S., Zhou, Y., Zhang, M., Chen, J., Xiang, Y., 2020. Expression of the immune checkpoint VISTA in breast cancer. Cancer. Immunol. Immunother. 69(8), 1437-1446.

How to cite this article;

Wajahat Masood, Abdul Wahab Ali, Afnaz Shaheen, Aeman Zulfiqar, Muhammad Saad Ul Haq, Syed Ali Raza, Ahsan Ashraf and Mehrullah. Protein biomarkers of feline mammary carcinoma. Veterinary Integrative Sciences. 2025; 23(1): e2025020-1-25.