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Review Article

Canine Mammary Tumors: Risk Factors, Prognosis and Treatments

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Abstract

Mammary tumors are the most common neoplasm in the female dog, with a median age of appearance between 9 and 11 years. They may appear as single or multiple nodules, and posterior mammary glands are more frequently affected than anterior glands. Both benign and malignant tumors may occur in the dog, and according to histological criteria, approximately 50% of the tumors are malignant. Mammary gland tumors tend to be heterogeneous in their pathological characteristics and clinical behavior. Different hormones and growth factors play a key role in the development of this neoplasm, however, the mechanism by which they influence tumor growth and their possible prognostic value are still under study. Besides, new therapeutic options for each particular tumor type are being developed. The aim of this article is to review pathological, prognostic and therapeutic aspects of canine mammary neoplasms.

Keywords: Canine, mammary gland, tumors, hormones, prognosis.

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Introduction

The mammary gland is a modified apocrine sweat gland, consisting in alveoli and ducts, surrounded by connective tissue, vessels and nerves. Bitches usually develop five pairs of glands, which are called thoracic (2 pairs), abdominal (2 pairs) and inguinal glands (1 pair). Mammary gland of dogs is the most common site for the development of benign and malignant tumors. Benign tumors are usually small, well circumscribed, firm on palpation, and they grow slowly, while malignant tumors are locally invasive, fixed to overlying skin or deep tissues, may be ulcerated and hemorrhagic, and they grow rapidly. Common sites of metastases are regional lymph nodes and lungs, although abdominal organs, bones, and brain can be also affected (Novosad, 2003).

Risk Factors

The etiology of mammary tumors remains unknown, although several risk factors for their development have been identified, including hormonal, genetic, and nutritional factors.

Hormonal Factors

Ovarian hormones, mainly estrogens and progesterone, are known to play a key role in the development of canine mammary tumors (CMT). Thus, the age at which the bitch is ovariectomized influences on the risk of developing them. According to the literature, bitches spayed before puberty have 0.5% of risk, while those spayed after one estrous cycle have an 8%, and those neutered after the second estrous have 26% of risk (Schneider *et al.,* 1969). Moreover, some reports have shown that estrogens and synthetic progestins, which are commonly used in veterinary practice, seem to enhance tumor formation (Rutteman 1992; Selman *et al.,* 1995; Stovring *et al.,* 1997). Recently, it was observed that levels of steroid hormones in serum and tissue homogenates were higher in bitches with malignant mammary tumors (MMT) compared to benign mammary tumors (BMT), indicating that steroid hormones could act as local growth factors in the malignant types, stimulating their proliferation (Queiroga *et al.,* 2005).

Several studies suggest that, in addition to circulating hormones, their receptors play a central role in mammary tumor formation. The presence of estrogen and progesterone receptors (ER and PR, respectively) in neoplastic mammary tissue has been demonstrated, and their expressions seem to be more frequent in BMT than in MMT (MacEwen *et al.,* 1982; Inaba *et al.,* 1984; Rutteman *et al.,* 1988; Donnay *et al.,* 1995, Martín de las Mulas *et al.,* 2005; Millanta *et al.,* 2005). However, a recent study has found that more than half of both BMT and MMT were negative for ER and PR, indicating lack of correlation between tumor type and ER and PR expression (Toniti *et al.,* 2009). These contradictory results may be related to different numbers and types of tumors analyzed in the studies.

Besides steroid hormones, other hormones and growth factors may influence mammary tumor development. Endogenous progesterone and synthetic progestins can increase the local production of Growth Hormone (GH) in both normal and neoplastic mammary glands of dogs (Selman *et al.,* 1994; van Garderen *et al.,* 1997). Growth Hormone leads to an increase in the concentrations of Insulin-like Growth Factor I (IGF-I), which stimulates mammary cell proliferation and act as a local growth factor promoting the development and maintenance of the tumor (Queiroga *et al.,* 2008). Recently, some researchers have proposed that Prolactin (PRL) could have a role in canine mammary tumorigenesis. A study showed that bitches with mammary tumors have higher serum PRL levels than healthy ones, and PRL levels in mammary tissue are greater in MMT than in BMT or normal glands, suggesting that neoplastic mammary tissue could be a source of PRL (Queiroga *et al.,* 2005). Moreover, Michel et al., (2012) observed that PRL receptor expression is lower in MMT than BMT, suggesting that the loss of this receptor may be a characteristic of tumor dedifferentiation.

Genetic Factors

In addition to hormonal factors, several studies have demonstrated that genetic alterations influence mammary tumor development, and certain breeds appear to display an increased predisposition to

cancer. There are variations between studies regarding the incidence of mammary tumors by breeds. Thus, in Czech Republic, the breeds reported to be at increased risk include the English Cocker Spaniels, Poodles, and Dachshunds (Zatloukal *et al.,* 2005). A retrospective study carried out in Japan has found that the incidence of MMT was lower in small breeds than in others (Itoh *et al.,* 2005). A recent study performed in Sweden has observed that the breeds most frequently diagnosed with this disease were the Leonberger, Irish wolfhound, Doberman, Welsh terrier, and English springer spaniel (Jitpean *et al.,* 2012). In Argentina, the English Cocker Spaniel, Pekingese and German shepherd have been reported as the breeds most frequently diagnosed with mammary tumors (Benavente *et al.,* 2013, Argentina, personal communication).

To determine the extent to which genetic factors may predispose to CMT, many genes have been investigated. For instance, proto-oncogene c-erbB-2 encodes for a transmembrane protein, called Epidermal Growth Factor Receptor 2 (EGFR-2 or HER-2), which normally regulates cell proliferation.

However, when it is mutated, HER-2 promotes neoplastic changes in the cells (Gutierrez and Schiff, 2011). Mutation and overexpression of HER-2 has been detected in both benign and malignant CMT (Rungsipipat *et al.,* 1998; Matsuyama *et al.,* 2001). In addition, HER-2 over-expression in MMT has been positively associated with nuclear pleomorphism, high histological grade and mitotic count (Dutra *et al.,* 2004). A recent study indicates that HER-2 contributes to increase the angiogenesis in mammary carcinomas (Carvalho *et al.,* 2013).

Another gene involved in mammary tumorigenesis is p53. The p53 tumor suppressor gene encodes a protein which normally regulates cell cycle and programmed cell death, and its mutations are known to contribute to the carcinogenic process of various organs, including the mammary gland (Greenblatt *et al.,* 1994). In CMT, p53 mutations have been detected in both benign and malignant types (Muto *et al.,* 2000; Lee and Kweon, 2002; Kumaraguruparan *et al.,* 2006).

Alterations of another tumor suppressor gene, called Breast Cancer 1 (BRCA1) have been observed in canine and human mammary cancer. A study has shown that BRCA1 is a nuclear protein, and the loss of its function could lead to an abnormal cytoplasmic distribution (Chen *et al.,* 1995). In the bitch, the absence of nuclear BRCA1 expression is associated with malignant characteristics of the tumors, including high proliferation index and absence of ERα expression, suggesting that it plays a role in the malignant behavior of this neoplasm (Nieto *et al.,* 2003). Abnormalities in the nuclear DNA content have been reported in CMT, being more frequent in malignant than in benign types (Hellmén *et al.,* 1988). In fact, DNA aneuploidy is associated with low survival rates of dogs with a diagnosed MMT (Hellmén *et al.,* 1993).

Nutritional Factors

Nutritional factors are of special interest due to the ability of adipose tissue to synthesize some steroid hormones. Adipose tissue is an important source of estrogens, owing to its aromatase activity, which converts androgens to estrogens. Increased mammary adipose tissue contributes to exposure of the gland to estrogens, which is known to promote mammary tumorigenesis (Simpson, 1996). Early studies in rodents have observed a close relationship between obesity and a high incidence of mammary tumors (Seilkop, 1995), and epidemiological studies in post-menopausal women have demonstrated that obesity is a risk factor for the development of breast cancer (Wu *et al.,* 1999).

According to Sonnenschein *et al.,* (1991), the risk of developing mammary tumors in the bitch is lower if they have been thin at 9-12 months of age. In addition, obesity at one year of age or one year prior to the diagnosis of the mammary tumor is related to an elevated incidence, and the intake of homemade meals, instead of commercial food, is also related to an increased risk (Pérez Alenza *et al.,* 1998).

Role of Cyclooxygenase Enzyme in Oncogenesis

There are two isoforms of prostaglandin synthases, also called cyclooxygenases - 1 (COX-1)

and 2 (COX-2). Cyclooxygenase -1 is a constitutive enzyme that is expressed in many tissues to ensure the synthesis of prostaglandins (PGs), which are necessary for physiological functions. In contrast, COX-2 is undetectable in most normal tissues, but it is inducible by growth factors, tumor promoters and inflammatory process (Smith *et al.,* 1996). In the past few years, numerous studies have demonstrated that COX-2 is involved in tumor development and progression. In fact, COX-2 expression has been found in several forms of human cancer, including carcinoma of colon, breast, and lung (Soslow *et al.,* 2000).

Similarly, COX-2 expression has been detected in several canine cancers, including prostatic carcinoma, oral melanoma, oral schamous cell carcinoma and mammary carcinoma (Mohammed *et al.,* 2004). Overexpression of COX-2 leads to the production of high concentrations of PGs, which have been reported to increase tumor cell resistance to apoptosis, enhance angiogenesis, increase tumor cell proliferation, and induce immunosupression (Fosslien, 2001). In CMT, COX-2 expression was first demonstrated in 2003 by Doré *et al.,* (2003). Further studies showed that MMT have a higher expression of COX-2 than BMT (Queiroga *et al.,* 2007, Dias Pereira *et al.,* 2009), and that this expression differs between histologic types in mammary carcinomas (Heller *et al.,* 2005).

Prognosis

The knowledge of different factors implicated in the prognosis and survival of bitches with mammary cancer, allows the clinician to establish an adequate treatment and to predict the potential recurrences or metastases. From a clinical perspective, advanced age at the moment of diagnosis is related to a poor disease-free and overall survival after mastectomy. Tumors larger than 3 cm in diameter, rapid and invasive growth, ulceration of the skin, fixation to underlying tissues, lymph node involvement, and presence of distant metastasis are also related to poor prognosis for the patient (Hellmén *et al.,* 1993; Yamagami *et al.,* 1996a; Pérez Alenza *et al.,* 1997, Ferreira *et al.,* 2009). Regarding pathological parameters, tumor type has been widely studied. Among malignant tumors, patients with sarcomas have the worst prognosis; among carcinomas, patients with anaplastic or solid carcinomas have lower survival rates than those with papillary, tubular or *in situ* carcinomas (Hellmén *et al.,* 1993, Misdorp *et al.,* 1999). Histological grading of malignant neoplasms also provides prognostic information. One of the most widely used grading systems in humans is the "Elston and Ellis method" (Elston and Ellis, 1991), which has been recently applied in canines (Karayannopoulou *et al.,* 2005). This last system includes three criteria: tubule formation, mitotic count, and nuclear pleomorphism, and the tumors are classified as well differentiated (grade I), moderately differentiated (grade II), or poorly differentiated (grade III), which is associated with the poorest prognosis (Karayannopoulou *et al.,* 2005; Goldschmidt *et al.,* 2011, Peña *et al.,* 2012). Besides, a study has shown that tissue invasion by cancer cells can predict the biologic behavior of the MMT, and the outcome of patients (Sarli *et al.,* 2002).

Cell proliferation index, which is usually determined by the cell cycle related marker Ki-67, has also been associated with prognosis, and high values of Ki-67 immunostaining have been positively correlated with the development of metastasis, low disease-free survival and overall survival rates (Peña *et al.,* 1998; Santos *et al.,* 2013).

Moreover, increased COX-2 expression is significantly correlated with HER-2 over-expression, tumor dedifferentiation and hence with a bad prognosis in bitches (Millanta *et al.,* 2006). More recent studies have found that COX-2 expression is associated with lymph node metastasis at the time of surgery, development of distant metastasis during the follow-up, and poor disease-free and overall survival (Lavalle *et al.,* 2009; Queiroga *et al.,* 2010).

In the last decade, special consideration has been given to hormone receptor expression and its value as a prognostic factor. Concerning steroid hormone receptors, in humans it is assumed that patients with ERα-positive breast cancer have better response to hormonal treatment and therefore, better prognosis, than patients without mammary tumor expression of ERα (Platet *et al.,* 2004). Similarly, a few number of studies in the bitch have

demonstrated that low levels of ERα are associated with increased tumor size, skin ulceration, lymph node involvement, and occurrence of metastasis during the follow-up, thereby indicating that ERα expression may be a marker of good prognosis (Nieto *et al.,* 2000; Martín de las Mulas *et al.,* 2005). More recently, the ERβ subtype has been detected in human breast cancer, and some authors have proposed a role for ERβ as a marker of good prognosis (Honma *et al.,* 2008). In the bitch, a study has demonstrated that ERβ is expressed in normal and neoplastic mammary glands, with higher expression in BMT than in MMT. Furthermore, the expression is higher in complex and mixed histological types of MMT (which are associated with a favorable prognosis) compared to simple types (Martín de las Mulas *et al.,* 2004), showing that ERβ expression may be a favorable prognostic factor.

Furthermore, in human breast cancer, the expression of PR was found to be more frequent in well-differentiated tumors than in poorly-differentiated ones, and a positive PR-status correlates with an increase probability of response to tamoxifen, and improves the outcome prediction over ER status alone (Bardou *et al.,* 2003). In the bitch, the population of PR in MMT is more abundant in those with clinical and histological parameters associated with favorable prognosis (Martin de las Mulas *et al.,* 2005), and dogs with MMT expressing PR had significantly higher survival rates than those lacking PR (Chang *et al.,* 2009). Recently, some authors observed that HER-2 over-expression in MMT was positively associated with indicators of poor prognosis in the bitch (Martin de las Mulas *et al.,* 2003; Dutra *et al.,* 2004). However, Ressel *et al.,* (2013) found that bitches with HER-2 over-expressing carcinomas did not present a poorer prognosis than those with HER-2-non-over-expressing tumors. Considering these controversial results, additional studies are required in order to investigate the significance of HER-2 over-expression in MMT. Concerning the relation between genetic alterations and prognosis, mutations in p53 tumor suppressor gene have been associated with significantly shortened survival time for dogs with mammary cancer (Wakui *et al.,* 2001; Lee *et al.,* 2004).

Conventional Therapies and New Treatment Strategies

Surgery remains the treatment of choice for all dogs with mammary gland tumors; exceptions include inoperable cases - such as inflammatory carcinomas and distant metastasis - and poor general condition. Several techniques have been described, including lumpectomy, simple mastectomy, regional mastectomy, and unilateral or bilateral mastectomy (Novosad, 2003).

The benefits of ovariectomy as a therapeutic option remains unsolved; some studies have shown improved survival time in bitches treated by mastectomy and ovariectomized than those treated by mastectomy alone (Misdorp 1988; Sorenmo *et al.,* 2000). Conversely, other studies have reported that ovariectomy at the time of mastectomy has no effect on the prognosis of CMT (Yamagami *et al.,* 1996b; Morris *et al.,* 1998). A recent study has demonstrated that ovariohysterectomy performed at the time of tumor excision reduces the risk of new tumors by about 50% among bitches with BMT (Kristiansen *et al.,* 2013).

Regarding adjuvant therapies, chemotherapy is sometimes indicated in bitches with invasive mammary tumors and high risk of recurrences or metastasis. However, no single chemotherapeutic protocol has been reported to be effective in the dog. Based on *in vitro* studies, doxorubicin has shown antitumor activity in CMT cell lines (Sartin *et al.,* 1993).

An *in vivo* study tested a chemotherapy protocol based on a combination of cyclophosfamide and 5 - fluouroucil after mastectomy, having a positive influence on disease-free interval and overall survival time of treated bitches (Karayannopoulou *et al.,* 2001). Another clinical trial comprised the treatment of bitches with MMT, with doxorubicin or docetaxel, but no benefit in overall survival rates was observed (Simon *et al.,* 2006).

Due to the hormonal dependency of this neoplasm, endocrine therapy is an option to consider. In women, hormonal therapy is a well-accepted adjuvant treatment and improves survival of patients with breast cancer. One of the most commonly used drugs is tamoxifen, a selective estrogen receptor modulator (SERM), which exerts

potent antiestrogenic actions in the breast (Platet *et al.,* 2004). However, in the bitch tamoxifen therapy is not advised due to its estrogen-like effects on reproductive organs, thus producing vulvar swelling, vaginal discharge, incontinence, stump pyometra, and signs of estrus (Novosad 2003; Tavares *et al.,* 2010). Further studies are necessary to find an effective drug with minimal side effects.

The presence of PRs in MMT opens the possibility to use PR antagonists as a post-surgery adjuvant therapy. There is one report about the use of a PR antagonist (aglepristone) in bitches with mammary carcinomas, but no beneficial effect was observed (Hermo *et al.,* 2009). A more recent study has shown that aglepristone has inhibitory effects on proliferation of PR positive canine mammary carcinoma cells (Guil-Luna *et al.,* 2011). Therefore, more studies are necessary to prove its efficacy.

In addition, Lombardi *et al.,* (1999) has tested hormonal therapy using Gonadotropin-Releasing Hormone (GnRH) agonists in bitches with hormone-dependent mammary carcinomas, showing a reduction in the size of the tumors, and beneficial effects in relapse-free survival.

Owing to the involvement of COX-2 in tumor development and progression, selective COX-2 inhibitors have been evaluated in the treatment of bitches with MMT. *In vitro* studies have demonstrated that certain COX-2 inhibitors (piroxicam and meloxicam) are able to inhibit cell proliferation in different canine cancer cell lines, including mammary carcinoma (Knottenbelt *et al.,* 2006). In addition, an *in vivo* study has shown that bitches with inflammatory mammary carcinoma treated with piroxicam have an improvement in disease stability and better outcome than those treated with traditional chemotherapy (De Mello Souza *et al.,* 2009). Despite the necessity of further studies, COX-2 selective inhibitors should be considered as part of an adjuvant therapy in dogs with mammary cancer.

Recently, antimetastatic effects of the peptide desmopressin (DDAVP) have been investigated. Desmopressin is a synthetic analog of the antidiuretic hormone vasopressin, and a selective agonist for the vasopressin 2 membrane receptor. This peptide has hemostatic and antidiuretic effects, and has been used in the management of diabetes *insipidus* (Manucci, 1997). Interestingly, DDAVP inhibited experimental lung metastasis of mammary tumor cells in a mouse model (Alonso *et al.,* 1999). In human breast cancer cell lines, DDAVP exhibits moderate antiproliferative effects (Iannucci *et al.,* 2011). Concerning studies on canines, Hermo *et al.,* (2008 and 2011) carried out two clinical trials in bitches with MMT to test the efficacy of perioperative administration of DDAVP after mastectomy. Desmopressin has a significant beneficial effect on disease-free period and overall survival time, and no side effects were observed in any of the patients. Thus, DDAVP seems to be a potent antitumor agent without evident side effects, and its use should be considered in the treatment of bitches with mammary cancer.

Conclusion

As mammary tumors are hormone-dependent neoplasms, the ovariectomy at an early age is crucial in order to prevent its development, and the necessity to reduce to a minimum the use of contraceptive steroids is also important.

The heterogeneity of mammary tumors makes them highly variable in their biological behavior, which generates the necessity of identifying factors with prognostic or therapeutic value for each particular patient and type of tumor.

In the last decade, advances in oncology research have allowed to discover previously unknown molecules involved in the carcinogenic process, and to develop therapies aimed at blocking these molecules. At present, the most promising therapeutic options are hormone receptor modulators, peptides with antitumor properties, and non-steroidal anti-inflammatory drugs for tumors expressing COX-2. Besides, more information about chemotherapeutic protocols is mandatory in order to find a safe and effective regimen that prolongs the survival time of bitches with mammary cancer.

Finally, in humans, monoclonal antibodies targeted to different molecules have been developed as new therapies to treat advanced breast cancer. Further investigation is required to assess the value of this type of therapy in the treatment of CMT.

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