Evaluation of clinical and laboratory investigation techniques of mammary gland tumors in the female dog: bibliographic study

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Abstract

In female dogs, the mammary gland tumors (MGT) and skin tumors are among the most common hyperplastic lesions of the animal species. Histologically, approximately 50-60% from all breast tumors are considered malignant lesions. Metastasis (circulating tumor cells from the primary tumor to the target organs – 60-80% lung dissemination) is the final step in the development of breast cancer, usually leading to death.

The study is systematized in two parts that include clinical investigation techniques (risk factors and prognosis factors) and paraclinical investigations (imaging, investigations used to establish the body’s functional status and pathological examination). A simple clinical examination seems to be insufficient, requiring a complex cases approach. In the present, if we refer to the results of different studies, cytological analysis does not lead us to a reliable prognosis. Using histopathological examination of lymphnodes and tumor is a source of additional information that will clarify the clinical prognosis.

The importance of prognostic factors are established in order to determine the risk of local or distant recurrence and the therapeutic way of choise to establish a strategy for monitoring the neoplastic process.

Key words: female dog, mammary tumors, metastases

Introduction

In female dogs, the mammary gland tumors (MGT) and skin tumors are among the most common hyperplastic lesions of the animal species (R.S. BRODEY & al. [1], E. DESTEXHE & al. [2], S. GIZIŃSKI & al. [3], E. MALICKA & al. [4]). It was found that the number of diagnosed cases has increased continuously, particularly in the case of malignant forms (K. ARNESEN & al. [5]). Histologically speaking, approximately 50-60% of the mammary tumors lesions are considered malignant (E.G. MACEWEN & al. [6], L. MOE [7]).

The metastases (circulating tumor cells from the primary tumor to the target organs) is the final step in the development of breast cancer, usually leading to death (W. WANG & al. [8]). These tumors can metastasize primary through blood vessels – by blood to lungs and by lymph vessels to regional lymphnodes and lungs (JH. HARVEY [9],).

Mammary tumors are easy to identify and metastases generally occurs from several months to several years after the discovery of the primary tumor.

Therefore, early and correct diagnosis of pulmonary metastases is of a considerable importance in establishing therapeutic measures, especially that 25%-50% of female dogs diagnosed with malignant breast tumors had metastases at the time of the fizical exam (CS. HEDLUND [10]).

Etio-pathogenesis. Etiology, its sources and its production methods are not fully known, although there are a number of factors which are known to induce mammary tumors in female dogs.
**Hormonal factors.** Progestational compounds administrated in order to control the estrus period is directly related to the occurrence of mammary tumors. These tumors are usually benign, although high doses and repeated administration favors their malignant transformation. These products therefore should be used with caution and always in a minimum dose, avoiding their repetition (J.A. MARTÍ [11]).

Endogenous estrogen and progesterone are considered to have a central role in the etiology of MGT. Estrogen and progesterone receptors are found in 50% of malignant MGT and 70% of benign MGT and also in the normal breast tissue (C. GOBELLO & al. [12]).

Tumors that don’t have hormone receptors behave more aggressively and are less differentiated than those who have these receptors. In addition, the presence of steroid receptors is not frequent in metastases, which could indicate an autonomous growth pattern (system). In humans, tumors rich only in estrogen receptors or estrogen/progesterone receptors regress after endocrine ablative therapy, but tumors which don’t have these receptors do not respond to therapy and are associated with a more reserved prognosis (C. GOBELLO & al. [12]).

Growth factors could play a role in normal mammary development and neoplastic tissues, including epidermal growth factor (associated with the presence of estrogen and progesterone receptors in the development TGM). In malignant mammary tumors, a direct correlation between epidermal growth factor and estrogen receptors was observed (J.A. Martí, [11]).

**False pregnancy (false lactations).** The studies undertaken by Donnay and col. (1994), has shown the fact that false lactation increases the risk of mammary tumors. The risk is even greater, as the female dogs are older (I. DONNAY & al. [13]). This risk was due to the food consumption that may contain carcinogenic elements and by hypoxia determined by abdominal swelling, resulting in the formation of carcinogenic free radicals. Repeated contact of these toxic products with the nipple epithelium induces pre-neoplastic lesions or aggravate existing injuries.

Prolactin is suspected of having a carcinogenic effect after the development of free carcinogen radicals in the mammary gland.

**Ovariectomy and ovariohysterectomy.** Ovariectomy is working on three levels to avoid malignancies. First to reduce the number of cells that can become cancerous, second in an indirect way to avoid false lactations and thirdly by ovariectomy the use of progestin to inhibit heat is eliminated (oestrus), (J.A. MARTI [11]).

No doubt, many oestrus cycles happened before sterilization are one of the most known risk factors for breast tumors. It is well known the protective effect of ovariohysterectomy on mammary tumors; since 1969 Schneider and col. Have confirmed this fact. They showed that the risk of developing breast cancer in spayed female dogs before the first heat cycle is 0.05%, while the risk increases to 8% if the ovariohysterectomy was performed after the first oestrus cycle, and reaches 26% after the second estrous cycle, because after 2.5 years from the ovariohysterectomy does not have a protective effect (R. SCHNEIDER & al. [14], S.J. ETTINGER & al. [15]).

**Viruses:** The existence of a virus responsible for mammary tumors has been demonstrated in other species and may not be entirely ruled out for dogs(murine mammary tumor virus). Murine mammary tumor virus (MuMTV) - was discovered in mice in which the virions flood in the mammary tumor cells and, as a feature, at the same time the cells are lactating; 40% of human breast tumors were detected being infested with DNA having a 95% MuMTV quantity) (J.A. MARTI & al. [11], D. CRÎNGANU [16]).

**Food:** In 1998, Perez Alenza and col. were interested in the influence of diet in the risk of developing mammary tumors. They obtained anamnetic data regarding reproduction Romanian Biotechnological Letters, Vol. 17, No. 6, 2012
and diet, and have studied the amount of selenium in retinol from the blood serum and the fatty acid profile from the subcutaneous adipose tissue on three groups of dogs (96 dogs with mammary tumors, 42 hospitalized dogs and 44 healthy dogs). The results have shown a significant role of obesity in developing mammary tumors, especially in 1 year old female dogs. (M.D. PEREZ ALENZA & al. [17])

In 2003, the same result was obtained by Philibert and col., which showed that obesity increases the risk of developing mammary tumors. Obesity in female dogs, aged 1 year old and within one year before diagnosis, was associated with a higher prevalence in developing mammary tumors and mammary dysplasia.

Thus, the overweight status influences the risk of developing mammary tumors. (J.C. PHILIBERT & al. [18]).

Cancer risk increases with diets that are home made and an important contribution to it is given by the red meat, especially beef and pork; the chicken meat has a lower contribution. (M.D. PEREZ ALENZA & al. [18]).

Other factors. Irregular oestrus, follicular cysts, persistent luteum body, the dimension of the puppies born in previous pregnancies, all these fertility problems have not been well documented in studies, but they were associated with an increased incidence of mammary tumors development; clinically speaking, this correlation is accepted (J.A. MARTI [11]).

Epidemiology – study of the risk factors:

Race influence. It seems that breast tumors are more common in pure breeds than in mixed breeds. The breeds in which the most mammary tumors lesions were registered are, in essence: Terrier Fox, Terrier, Cocker, Caniche, Pudel, Labrador, English Setter and Pointer (S. MAUFRE [19], A. MULLER & al. [20]). Researchers in this field are adding the fact that this tendency is due to the popularity of these breeds, but also due to the hormonal medicamentation which is frequently used in contraceptive purposes. These small and average breeds are often controlled by their owners, which allows an early detection of nodules. (S. MAUFRE [19], A. MULLER & al. [20]).

Age influence. Various studies regarding the impact of age in developing MGT, showed that the incidence increases as years passes and the maximum frequency is found between 9 and 11 years. On the other hand, researchers have attempted to correlate different age categories lesions such as mammary dysplasia which usually occur at younger ages, between 2 and 4 years old. Generally, before 5 years, most commonly diagnosed tumors are benign and after 6 years increases the incidence of malignant tumors (M.D. PEREZ ALENZA & al. [21]).

Gender influence. Females are almost exclusively affected by this type of tumor. However, studies have shown that 1% of these tumors are observed in males (J.P. MAGNOL & al. [22], M. MIALOT & al. [23], M. LAGADIC & al. [24]).

Clinical signs – prognostic factors:

Locating the tumoral process. In 65-70% of cases, tumors are present in M4 and M5 caudal gland (G.R. RUTTEMAN & al. [25]). Identifying and defining the affected glands is an indispensable step, which is subject to further clinical examination of the lymphatic system.

In a study conducted in 2009, Sontas BH. and col., examined a number of 133 tumorized mammary gland and found that in 22.5% of these cases the thoracic gland were involved (M1, M2), in 30.1% the abdominal gland (M3, M4) and in 47.4% the inguinal gland (M5) (BH. SONTAS & al. [26]).

Tumor growth rate. During the consultation, the doctor must seek out and determine, by questioning the owner, the onset data and the growth rate. But this parameter is not always reliable.
The rapid growth seems to be associated with a poor prognosis, but a study by Perez Alenza and col. Conducted in 1997, does not highlight a significant link between tumor growth rate and survival female dog (M.D. PEREZ ALENZA & al. [27]). This parameter may be interesting to study, but this remains an uncertain criteria.

*Tumor growth mode.* The practitioner should also try to clarify the tumor mobility compared with the superficial and deep adjacent tissue (K. SORENMO [28]). A study made by Yamagami and col. Conducted in 1996, showed that increased tumor infiltration and adhesion to skin and / or surrounding tissues was accompanied by a poor prognosis (with a survival rate of approximately two years) (T. YAMAGAMI & al. [29]).

Another study showed no significant relationship between mthe growth mode and the survival rate. Therefore, further studies should be undertaken in order to determine whether the tumor growth mode can be considered as being a prognostic factor (M.D. PEREZ ALENZA & al. [27]).

*Tumor dimension.* Many studies have shown that the survival rate and metastasis rate were significantly correlated with the mammary tumor size. It was demonstrated the fact that higher the tumor is, it greatly reduced the life expectancy of the animal is. (SC. CHANG & al. [30], J.C. PHILIBERT & al. [18], G.R. RUTTEMAN & al. [25], T. YAMAGAMI & al. [29]). However, we must be aware that small tumors can be malignant. Similarly, a large tumor may be benign (J.P. MAGNOL & al. [22]).

*Tumor ulceration.* Ulceration of the skin which covers the mammary tumors is a parameter commonly associated with other prognosis, including increased risk of metastasis (E. HELLMEN & al. [31], SE. LANA & al. [32], L. PENA & al. [33], M.D. PEREZ ALENZA & al. [27]). However, this is not a clear prognostic factor (Fig 1).

The presence of ulceration on a small tumor is therefor an interesting criteria for clinical prognosis, because it could indicate a sign of malignancy easily identifiable.

![Caniche, 10 years old, F. Oversized mammary tumor on the left mammary chain – M3, with an ulceration presence (original) – Clinical examination was made at the Faculty of Veterinary Medicine – Bucharest](image)

Degree of regional expansion. It matches the clinical examination of the affected nipple) and the associated lymph. Most mammary tumors are of epithelial origin with lymphatic dissemination.

The clinician must carefully palpate the lymphatic drainage lymphnodes (axillary and/or superficial inguinal) and follow their mobility to neighboring tissues. Indeed, the
metastatic process is clinically characterized by adenomegaly and sometimes by adhesion to the subcutaneous tissue. (S.C. Chang & al. [30], E. Hellmen & al. [31], GR. Rutteman & al. [25]).

But the presence or absence of lymphadenopathy is a concept that needs to be clarified. Adenopathy can also be an evidence of an inflammation (lymphadenitis). Only the histopathological examination of the lymph node can confirm or infirm the presence or absence of metastatic adenopathy.

Similarly, the lack of adenopathy does not imply the absence of metastases. (S.C. Chang, & al. [30], E. Hellmen, & al. [31], G.R. Rutteman & al. [25]).

All studies regarding metastatic dissemination agree the fact that the presence of loco-regional lymphnodes metastases are locally strong prognostic factor. The presence of lymphnode metastases is associated with lower survival prognosis. Relapse rate of metastasis was of 80% during a six months period if the female dogs showed lymphnode metastases at the moment of diagnosis, compared with 30% within two years if there weren’t identified any from the absence of lymphnode metastases in the moment of diagnosis (M. Vincent [34]).

**General extenton degree.** After the regional lymphnodes, mammary tumors are metastasize especially in lungs (60-80%) – (Fig 2), but also in other organs: deep visceral lymphnodes, adrenal glands, kidneys, heart, bones, liver, brain, eyes, nose, spleen, uterus, serouses (A. Fontbonne & al. [35], M. Lagadic & al. [24], A. Muller & al. [20], K. Sorenmo [28]). Occasionally, skin can also be a metastasis place of canine mammary tumors, causing skin carcinomatosis (A. Muller & al. [20]).

**Fig 2.** Cocker, 14 years old F. Necropsic image - the presence of multiple metastases in the lungs (original) – The Pathological Anatomy Laboratory of the Faculty of Veterinary Medicine – Bucharest

**Paraneoplastic syndromes.** The three main paraneoplastic syndromes found in breast cancers are: fever, disseminated intravascular coagulation (DIC), hypertrophic osteopathy (also known as the pulmonary hypertrophic osteopathy disease, Marie syndrome, acropachia or Cadiot-Ball syndrome) is a secondary bone disease (most cases are associated with primary or secondary lung affections - neoplastic metastases), characterized by the occurrence of an irregular, painful generalized periosteal proliferation of the distal extremities of one or four limbs. It should be noted that hypercalcemia is rare in these cases (F. Cohn-Bendit [36], N. Tudor & al. [37]).

**Diagnosis.** In addition to clinical examination it is recommended a profoundly exploration, including laboratory investigations: imaging (radiographs, ultrasound, computed tomography-CT, magnetic resonance imaging-MRI and thermographic examination),
investigation which are aimed on the functional body condition (biochemical and hematological analysis and electrocardiographic evaluation-ECG) and pathological examination (macroscopic, cytology and histology).

Among commonly used imaging methods in identifying lung metastases X-ray, computed tomography (CT) and magnetic resonance imaging (MRI), are included. (C.S. HEDLUND [10], P.Y. BARTHEZ & al. [38], D. BAUMANN & al. [39], S. NEMANIC & al. [40], J.F. SCHAFFER & al. [41])

Radiological examination. The Rontgen diagnosis is a complementary method of clinical examination, which uses the X-beam radiation in a diagnose purpose on animals. (C. VLĂGIOIU & al. [42]).

This technique is indicated if there is a risk of metastatic disease. Chest radiographs should be evaluated for pulmonary metastases and mediastinal lymph adenopathy (Fig 3).

![Fig 3. Caniche, 10 years old F. X-ray examination shows the presence of multiple areas of pulmonary radio-absorption (lung metastases) (original) - The X-ray laboratory of the Faculty of Veterinary Medicine - Bucharest.](image)

The metastatic spread to the pleura was also reported as being possible in the case of female dog (M. ROBBINS [43]). If the neoplasm involves caudal mammary glands, the sublombary lymphnodes should be evaluated using X-ray and/or ultrasounds. Radiologically speaking, pulmonary metastases can be described as well defined nodules or with a poor delimitation, with or without pleural effusion.

Those presenting a poor separation, with or without pleural effusion, does not represent a clear evidence of pulmonary tumor aggression (M. ROBBINS [43]).

The research was highlighted the distribution of metastatic lesions in terms of pulmonary X-ray, resulting the following classification: unique metastases, multiple metastases, interstitial ± peribronhic nodular infiltrate. (M.VINCENT [34]).

The mineralization of breast lesions was more frequently associated with benign lesions, than with the malignant ones.

Mineralization can be seen on a survey X-rays, along abdomen in the ventral zone, as well as distal acoustic shadows in ultrasound (R. O'BRIAN & al. [44]), (Fig 4).
In another study, 29% of female dogs radiologically diagnosed without visible metastases have later developed lung metastases. Metastases have developed within 6 months after surgical removal of the tumor (J.P. MAGNOL & al. [22]). But conventional X-ray is less sensitive in detecting metastatic lesions with a diameter of 6-8 mm/7-9 mm (K. SORENMO [28], S. NEMANIC & al. [40]). It is preferred the use of the X-ray because it is a rapid and profitable survey method and does not require general anesthesia (C.S. BAPTISTA & al. [45]).

X-ray examination is mainly used to track the occurrence of any metastasis in the organ of choice. For this the most common examinations are, not only the examination of the thoracic cavity (contribution made by X-ray) but also the examination of the abdominal cavity, the most common being ultrasound is desirable (C.S. FARROW [46]).

**Ultrasound examination.** Regarding evaluation, the use of ultrasounds for evaluating the primary breast neoplasms shows us a proper representation of cancer tissue composition. In addition, using Doppler ultrasound the vascularization of the mass tumor is demonstrated. However, the degree of local invasion is not accurately portrayed using the ultrasound. Ultrasound is usually used to identify the metastases located in the abdomen and sometimes at chest level too. Ultrasound evaluation of mediastinal adenopathy is more effective than X-ray (R. O'BRIAN & al. [44]).

A study undertaken by Baștan A. and col. in 2009 has indicated the fact that ultrasound is extremely useful in evaluating canine mammary tumors, especially in determining the size of the tumor, the tumor shape, the tumor irregular margins, the tumor echostructure, the internal echogenicity and acoustic transmission (A. BASTAN & al. [47]).

MRI, and also CT are highly "sensitive" imaging technics in detecting the pathology at the body level (metastasis detection that have not been highlighted by other diagnostic methods), but has some drawbacks (the lack of specialized equipment for veterinary hospitals and the high prices).

In detection, assessment and management of mammary tumors, magnetic resonance imaging (MRI) is emerging as redoubtable technique in detecting, diagnosing and monitoring the anomalies (M.D. SCHNALL [48]).
In a retrospective study on 18 dogs with pulmonary metastases of MGT there were detected small pulmonary nodules.

CT scan detects nodules sized from 1 mm, compared with radiography that detects nodules from 7 mm to 9 mm. (S. Nemanic & al. [40])

Although CT is considered a more sensitive method than radiography for detecting lung metastases, there is a risk of false positive or false negative (S. NEMANIC & al. [40], DJ. WATERS & al. [49], F.V. COAKLEY & al. [50]).

**Thermographic examination.** Thermographic examination is a non-invasive investigation method and diagnosis, based on the determination of calorific radiation emissions produced by normal tissue or a lesioned one. It is known the fact that metabolic activity of tumor tissue is increased compared to normal tissue. It is also known that tumor angiogenesis is an essential factor for tumor growth and metastasis development. Speeding up metabolism and angiogenesis are two factors that induce a local temperature rise in mammary tumors. (M. MILITARU & al. [51], J. MOCANU & al. [52])

**Investigations aimed the functional body condition.** Biochemical and hematological tests aimes tracking the following parameters: hepatic (GPT, AP, bile acids), renal, pancreatic, haemostatic, including calcium and urinalysis. In most of these cases, the results of these test are normal (J.A. MARTI [11]).

Hypercalcemia is a rare paraneoplastic symptom in mammary carcinoma and in this case the liver functionality is very useful in research of liver metastases (rarely associated with liver failure). Examination of these functional parameters of the body is of interest in the context of pre-anesthetic assessment before surgery (K. SORENMO [28]).

ECG examination before submission the geriatric animal to surgery is required in order to determine the cardiac functional parameters (J.A. MARTI [11]).

**Pathological examination.** Diagnosis of mammary tumors in the female dog and woman can be confirmed by observation (suspicion diagnosis) and/or histopathological examination (certainty diagnosis), implementing deep biopsis for detecting the neoplastic emboli from dermal lymphatic vessels. In most cases, the clinical and pathological exam are the same but some patients may present only clinical signs or histopathological changes. (M.D. PÉREZ-ALENZA & al. [53], M.D. PÉREZ-ALENZA & al. [54])

The presence of inflammatory edema or hardened areas, with rapid onset, which affects the nipple and adjacent skin should lead to perform fine needle aspiration or biopsy. Cytological exam is rarely used in examining mammary tumors and it is useful in excluding an inflammatory process (and therefor the differential diagnosis area is narrowed).

However, the study undertaken by Susaneck, during which he made fine needle aspiration on seven animals with mammary tumors, has always conducted to the carcinoma diagnosis, while the study made in 2001 by Pérez Alenza and col. during which 33 animals were cytological examined has shown that only 15 of them were presenting neoplastics (S.J. SUSANECK & al. [55], M.D. PÉREZ-ALENZA & al. [54]). Cytological diagnosis can not be used without further investigations to confirm the diagnosis, as certainty, because it can not provide information regarding the dermal lymphatic vessels involved or regarding the histological type. Puncture cytology may be helpful in assessing lymphnodes that are suspected of metastases (Fig 5), (S.E. LANA & al. [32]).
Accurate diagnosis is the histopathological one. It is performed on skin biopsies or after removing the affected mammary chain. Due to the histopathological analysis of the frotium a malignant tumor proliferation is observed, composed of glandular cells, pleomorphic, often poorly differentiated, isolated, sometimes arranged in small groups or as lobules. Dermis, hypodermis and epidermis can be stubbly.

An infiltration of the cutaneous lymphatic vessels is also shown by the numerous lymphatic neoplastic embolism in the skin. Images of the lymphatic penetration are sometimes visible. (Fig 6) (A. MULLER & al. [20], Z. ALHAIDARI & al. [56], P.J. GINEL & al. [57]).

Fig. 5. Cocker, 14 years old F. Cytological examination of the mammary gland tumor, ob * 4. Diagnosis is of complex mammary carcinoma type (original) - The Pathological Anatomy Laboratory of Faculty of Veterinary Medicine - Bucharest.

Fig. 6. Cocker, 14 years old F. Histopathological examination of the mammary gland tumor, ob * 4. Diagnosis is of complex mammary carcinoma type (original) - The Pathological Anatomy Laboratory of Faculty of Veterinary Medicine - Bucharest.
The diagnosis should always include a histopathological examination of the nipple and the excised tumor, because depending on the tumor type, treatment and prognosis can vary widely (G. BRASSART [58]).

Conclusions

1. On female dog, the prognostic factors are established in order to determine the risk of local or distant recurrence, to choose the most appropriate therapeutic way and to establish the monitoring strategy. Animal survival in the best conditions is a major concern of the owner, thus it requires a close collaboration between the owner, the clinician and the pathologist. Prognostic factors allows the veterinarian to answer to some of this concerns.

2. Currently, clinical and histopathological information are an essential part of prognostic orientation. When making a clinical examination, the primary tumor characteristics (growth rate, infiltration, tumor size, presence of ulceration) and the presence of metastases are important prognostic criteria.

3. A simple clinical examination seems to be insufficient, requiring a complex cases approach. In the present, if we refer to the results of different studies, cytological analysis does not lead us to a reliable prognosis. Using histopathological examination of lymphnodes and tumor is a source of additional information that will clarify the clinical prognosis.

4. The multiple investigations applied in different techniques, are designed to provide safety in the establishment of a diagnosis.

5. In current conditions, with detection and correct diagnosis, in applying a suitable protocol, many of canides breast tumors can be treated and healed, improving the life quality of the patient.

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