Factors influencing the incidence and prognosis of canine mammary tumours

Factors relating to the incidence of canine mammary tumours are reviewed. Increased age, intact status or ovariectomy after 2·5 years of age, as well as progestagen treatment, can all lead to an increased risk of mammary neoplasia in the bitch. In addition, obesity early in life, and a habitual diet based on home-made food (rich in beef and pork, and poor in chicken) as opposed to commercial food, are also associated with the occurrence of mammary tumours. Other aspects related to incidence are also discussed. Increased age at diagnosis, invasive growth (fixed to adjacent tissues), large tumour size, ulceration of skin, and axillary or inguinal node involvement are clinical parameters associated with a low chance of survival after surgical excision of mammary tumours. Histological typing and grading of the tumour allows the establishment of a prognosis, which is poor where there is tumour proliferation as measured by S-phase fraction determination and Ki-67 immunostaining.

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INTRODUCTION

The clinical presentation of canine mammary tumours (CMTs) is variable: they occur either as single or multiple nodules, and, if multiple, can be of the same or different histological types. The clinical behaviour ranges from well-circumscribed nodules with a stationary growth to large and sometimes ulcerated nodules, which grow rapidly and become fixed to adjacent tissues, or have other signs of malignancy, such as the 'inflammatory carcinoma' (Fig 1). The proportion of tumours that are malignant ranges from 41 to 53 per cent (Brodey and others 1983). This figure may not be accurate because the owner will have their attention drawn to a tumour that has produced systemic signs due to distant metastases, but an asymptomatic tumour may go unrecognised. The target organ for metastases is the lung, although distant metastases can occur less frequently in the abdominal cavity (liver, kidneys and adrenal glands), brain, eyes and bones.

The initial diagnosis of CMTs is based on clinical features; fine-needle aspiration may be useful (Hellmén and Lindgren 1989) but, in most cases, histology is necessary for an accurate diagnosis (Allen and others 1986). In order to establish the clinical prognosis, plan treatment, and evaluate the results of treatment, clinical staging must be performed using the TNM system (Owen 1980).

In the present study, factors influencing the incidence and, thus, the potential prevention of these neoplasms are reviewed, together with recent findings relating to prognosis.

INCIDENCE

Mammary tumours are the most frequent neoplasms in the bitch (Moulton 1990). The incidence is extremely high in those areas where early ovariectomy of bitches

FIG 1. 'Inflammatory carcinoma'. These *mammary tumours* grow rapidly (days or weeks) invading the skin, with pain and inflammation

(before two years of age) is not a routine clinical practice, such as in Spain and some other European countries. The annual incidence rate in intact female dogs was estimated to be approximately 260/100,000 animals at risk according to a study carried out in California (Dorn and others 1968). Middle-aged bitches are primarily affected (mean nine to 11 years of age) and an increase in incidence begins at approximately six years of age. Mammary dysplasias occur in younger bitches (two to four years of age). The development of malignant tumours before five years of age is rare, and if tumours occur at this age, they are usually benign in nature.

A higher incidence in hunting breeds compared with the boxer and Chihuahua has been reported (Brodey and others 1983, Theilen and Madewell 1987), while other studies found lower incidences among mixed breeds compared with pure breeds (Dorn and others 1968, Kurzman and Gilbertson 1986, Karayannopoulou and others 1989). In one study, no clear breed predisposition was found (Pérez Alenza and others 1998). This lack of unanimity is probably due to the different canine populations used in the studies.

CMTs occur mainly in female dogs, although the incidence in males has been estimated (0 to 2·7 per cent) (Brodey and others 1983).

RISK FACTORS

CMTs can develop according to a number of influences. Hormonal factors are the most widely studied, and play a principal role in mammary carcinogenesis in dogs.

The role of sexual steroid hormones – oestrogen and progesterone – in the pathogenesis of CMTs is well established (Rutteman 1990). Evidence is supported by a reduction in risk following ovariectomy, if this is performed early in life, at before 2·5 years of age (Schneider and others 1969, Taylor and others 1976). However, ovariectomy after a mammary tumour has developed does not have a significant effect on the progression of malignant disease (Yamagami and others 1996, Morris and others 1998). Furthermore, oestrogen and progesterone receptors have been identified in mammary tumours (MacEwen and others 1982, Mialot and others 1982, Elling and Ungemach 1983, Monson and others 1987, Sartin and others 1992, Donnay and others 1995). These are present in higher proportions in normal mammary glands and benign tumours than in malignant ones (Mialot and others 1982, Rutteman and others 1988b, Donnay and others 1995), and metastatic sites of mammary tumours are frequently receptor negative. Thus, steroidal hormones act on target cells during the early stages of mammary carcinogenesis, but seem to lose their stimulatory effect during the latter stages of the disease.

Other growth factors could play a role in the development of normal and neoplastic mammary tissues, including epidermal growth factor (EGF), transforming growth factors (TGFs) (Nerurkar and others 1987, Rutteman and others 1990) and parathyroid hormone-related protein (Okada and others 1997). EGF and TGFs are associated with the presence of oestrogen and progesterone receptors in CMTs; in malignant tumours, a direct correlation was observed between EGF and oestrogen receptor expression (Donnay and others 1996). Furthermore, abnormalities in pituitary hormones seem to play their own role: growth hormone (GH) excess induced by progestins could influence mammary tumour development (Rutteman 1990, Selman 1994), which may occur by the stimulated proliferation of susceptible and transformed mammary epithelial cells (van Garderen and others 1997). Prolactin receptors have been found in mammary tumours, although the role of this hormone remains unclear (Rutteman 1990).

Ovarian hormones are not the only agents known to influence mammary tumour development. Combinations of oestrogens and progestins (medroxyprogesterone acetate), used in veterinary practice to prevent oestrus or to treat pseudopregnancies, may increase the incidence of mammary neoplasia (Rutteman 1990, Zanninovic and Simcic 1994, Stovring and others 1997). Medroxyprogesterone acetate and proligestrone administration in healthy dogs both result in an overproduction of GH, suppression of the adrenal cortex and the development of dysplasias and benign mammary tumours (Selman 1994). Perhaps similar mechanisms are involved in dogs with spontaneous mammary tumours previously treated with progestins.

Regarding other factors, such as the number of pregnancies, age at first full term pregnancy (Taylor and others 1976) or disorders of oestrous cycles (Schneider and others 1969, Else and Hannant 1979), the results of various studies are contradictory, although it seems that these variables do not add any significant risk to the development of CMTs (Theilen and Madewell 1987). Several authors have observed that pseudopregnancy has no effect on the development of CMTs (Brodey and others 1966, Fidler and others 1967, Morris and others 1998), while others found a positive relation (Donnay and others 1994). If some of these reproductive aspects are associated with the incidence of CMTs, the relation is probably weak in view of the disparity of the results to date.

Virus-associated c-particles have been described in conjunction with CMTs in one study (Watrach and others 1978); however, a viral aetiology has yet to be proved, and no other research confirms this isolated finding.

In the past decade, the effect of other factors on canine mammary tumorigenesis has been studied, and nutritional factors are of especial interest in view of their role in the development of human breast cancer. In a study of ovariectomised female dogs, obesity at a young age increased the risk of mammary tumours, and the proportion of fat in the habitual diet of affected dogs was lower than in controls (Sonnenschein and others 1991). In a

recent epidemiological study (Pérez Alenza and others 1998), obesity at a young age and one year before diagnosis, and the intake of home-made food (compared with that of commercial foods), have been related to a higher incidence of tumours and dysplasias. Other factors associated with the presence of CMTs are a high intake of beef and pork and a low intake of chicken. Also, serum retinol concentration has been lower in affected dogs than in controls. However, the proportion of macronutrients (fat, protein and carbohydrates) does not appear to be significant. It seems that obesity at a young age (when the early carcinogenetic events occur in canine mammary glands) is related to a higher incidence of CMTs, but whether this is a direct effect or not remains to be established. Also, the habitual diet in adult female dogs influences the occurrence of CMTs; however, the role of retinol, and the intake of food constituents, such as beef and pork, has yet to be elucidated.

PROGNOSIS

Owing to the high incidence and variability of CMTs, it is important for the clinician to know which factors allow the establishment of an initial prognosis and adequate treatment. Furthermore, prediction of post-surgical behaviour is necessary, and several clinical and pathological parameters could be used to predict the potential occurrence of recurrences and/or metastases.

There are several univariate follow-up studies on CMTs that offer information about prognostic factors. However, these parameters should be evaluated using multivariate analyses, in order to know whether or not a factor could predict prognosis independently. There are only two multivariate analyses on the prognosis of CMTs (Hellmén and others 1993, Peña and others 1998). Unfortunately, some known prognostic factors in human breast cancer have been adopted as valid in veterinary medicine, without knowledge of their real influence in dogs.

Clinical factors

Increased age at diagnosis has been associated by many authors with a shorter disease-free survival (DFS) and overall survival (OS) following surgical excision (Bostock 1975, Hellmén and others 1993, Pérez Alenza and others 1997, Peña and others 1998). It is an independent factor (Hellmén and others 1993, Peña and others 1998) and strongly related with a poor prognosis for dogs with mammary tumours.

One author has reported a poorer prognosis for the German shepherd dog in comparison with other breeds (Withrow 1975).

Reproductive variables, such as ovariectomy once the tumour has developed, number of pregnancies and age at first full term pregnancy, oestrous cycle features and pseudopregnancies, do not affect the prognosis of mammary tumours (Schneider and others 1969, Else and Hannant 1979, Hellmén and others 1993). The presence of short oestrous cycles and a low number of oestrous cycles have been related to shorter OS and DFS in univariate analyses (Pérez Alenza and others 1997), but this has not been confirmed using a multivariate analysis (Peña and others 1998).

In human breast cancer, a high content of oestrogen receptors in a tumour is associated with a better prognosis and allows hormonal treatment after surgical excision. In dogs, the presence of oestrogen receptors in a malignant mammary tumour also seems to relate to a better prognosis, although more studies would be necessary to confirm this.

Rapid and invasive growth, and large tumour size are clinical parameters that have been associated with a poor prognosis for CMTs in many studies (Bostock 1975, Misdorp and Hart 1976, Else and Hannant 1979, Kurzman and Gilbertson 1986, Hellmén and others 1993, Yamagami and others 1996, Pérez Alenza and others 1997, Peña and others 1998). Thus, an invasive growth with fixation to skin and/or underlying tissues (which should be confirmed by histology) and a large tumour size are useful objective features often indicating a poor prognosis. However, rate of growth is subjective (information from the owner) and gives less prognostic information (sometimes the owner does not see a small nodule in a longhaired dog). Ulceration of the skin has been associated with malignancy (Hellmén and others 1993, Pérez Alenza and others 1997, Peña and others 1998) and is considered an independent factor strongly associated with a poor prognosis.

In one study (Misdorp and Hart 1976), lymph node involvement was not related to prognosis of CMTs, although, in the majority of the studies where this was investigated (Fidler and others 1967, Hellmén and others 1993, Yamagami and others 1996, Pérez Alenza and others 1997, Peña and others 1998), a positive association was observed.

In addition to the pattern of tumour growth (T) detailed above, an assessment of nodal (N) and distant metastases (M) is used by the clinician to establish a prognosis.

Pathological factors

There are several histological classification systems for CMTs (Fowler and others 1974, Gilbertson and others 1983). The former WHO classification, which is the one accepted by the majority of pathologists (Hampe and Misdorp 1974), has recently been substituted by a new one (Misdorp and others 1999). The value of these systems is established regarding the prediction of tumour behaviour following surgical excision. The diagnosis of mammary tumours sometimes uses individual pathologists' criteria, and thus the proportion of canine malignant mammary tumours varies from 26 to 73 per cent according to different studies. Nevertheless, sarcomas are described as the tumours with the worst prognosis (Else and Hannant 1979, Kurzman and Gilbertson 1986, Hellmén and others 1993), and solid carcinomas have been shown to have lower survival times than papillary or tubular carcinomas (Bostock 1975). Mixed malignant tumours and squamous cell carcinomas are likewise considered to carry a poor prognosis. Histological types associated with a better

prognosis are adenocarcinomas in general (complex types more so than simple) and carcinomas in situ.

Using a previously established grading system for human breast carcinomas (Bloom and Richardson 1957, Scarff and Torlioni 1968), histological grading of neoplasms in dogs can provide additional prognostic information. Three degrees of histological malignancy can be recognised: grade I (well differentiated with no vascular or lymphatic invasion), grade II (moderately differentiated neoplasms with no invasion) and grade III (poorly differentiated tumours with or without invasion). Even though there is no unanimity for establishing a definitive grading system (Misdorp and Hart 1976, Kurzman and Gilbertson 1986), it is generally accepted that grade III is associated with the worst prognosis. A specific grading system needs to be established for CMTs using follow-up validation.

DNA analyses using flow cytometry allow the DNA content of tumours and the ploidy status to be established; the incidence of aneuploidy in malignant tumours is higher (48 to 62 per cent) than in benign tumours (Rutteman and others 1988a, Hellmén and others 1993, Pérez Alenza and others 1995) and is related to a poor prognosis. Likewise, the mean nucleolar organiser region count in silver-stained sections (AgNOR) is associated with a poor prognosis in dogs surgically treated for mammary neoplasms (Lohr and others 1997). S-phase fraction (SPF) measures the tumour proliferation, and a high SPF determined with flow cytometry has been found to be independently correlated with an adverse prognosis using a multivariate analysis (Hellmén and others 1993).

Recently, the determination of tumour proliferation indices by immunohistochemical detection of the cell-cycle related marker Ki-67 (Fig 2) has been related to prognosis of CMTs using a multivariate analysis: increased Ki-67 immunostaining was an objective prognostic parameter in predicting the postsurgical behaviour of canine mammary neoplasms (Peña and others 1998).

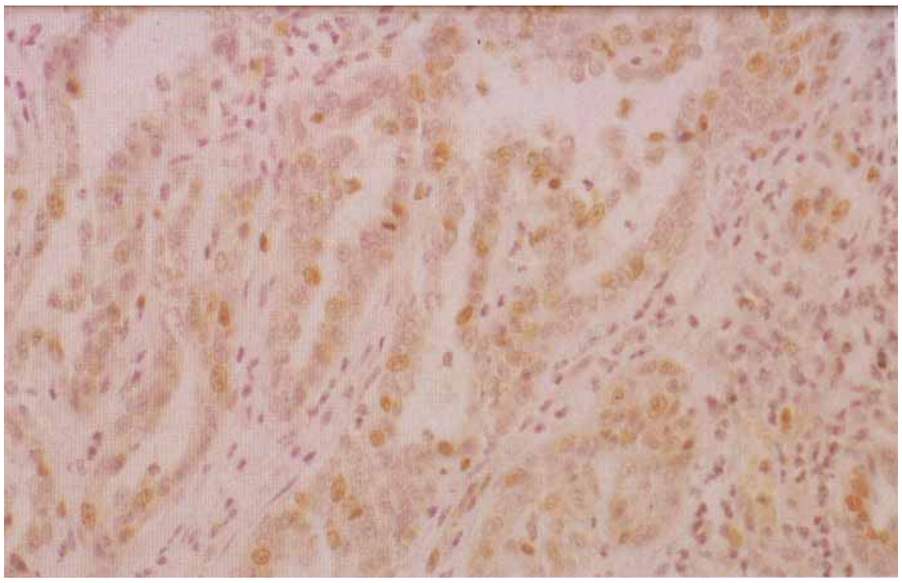

FIG 2. Ki-67 immunostaining in a mixed malignant mammary tumour

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