J. Comp. Path. 2005, Vol. 133, 246–252

ELSEVIER

www.elsevier.com/locate/jcpa

Histological Grading and Prognosis in Dogs with Mammary Carcinomas: Application of a Human Grading Method

M. Karayannopoulou*, E. Kaldrymidou†, T.C. Constantinidis‡ and A. Dessiris*

**Department of Clinical Studies and †Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, St Voutyra 11, 546 27, Thessaloniki, and ‡Laboratory of Hygiene and Environmental Protection, Medical School, Democritus University of Thrace, Greece*

Summary

The human "Elston and Ellis grading method" was used in dogs with mammary carcinoma to examine its relation to prognosis in this species, based on a 2-year follow-up period. Of the 85 cases examined, 27(31.8%) had well-differentiated (grade I), 28 (32.9%) had moderately differentiated (grade II) and 30 (35.3%) had poorly differentiated (grade III) carcinomas. Two years after mastectomy, significant differences in survival between cases with different tumour grade were found; thus, survival was worse in dogs with grade III carcinomas than in those with grade II ($P<0.05$) or grade I ($P<0.001$) tumours. However, in dogs with simple carcinomas which had a less favourable prognosis than that of other carcinomas ($P<0.001$), there was no significant difference in survival between grade II and grade III cases ($P=0.878$), both having a very poor prognosis. Undifferentiated (grade III) carcinoma cases had a 21-fold increased risk of death as compared with differentiated (grade I and II) carcinoma cases. An increased risk (about 10-fold) was also associated with undifferentiated simple carcinomas as compared with differentiated ones. The predictive value of histological grade was not influenced by tumour size or age of the dog at mastectomy; nodal metastasis, however, worsened the prognosis ($P<0.001$). Routine use of this human grading method would help the clinician to make a more accurate prognosis in the interests of post-surgical management in dogs with mammary carcinomas.

Keywords: carcinoma; dog; grading of tumours; histological grading; mammary carcinoma; tumour

Introduction

Mammary tumours are the most common tumours in the female dog (Kitchell and Loar, 1997; Morrison, 1998; Misdorp, 2002; Davidson, 2003). Reports of the occurrence of malignant forms vary from 26 to 73% (Pérez Alenza *et al.*, 2000), carcinoma being the most common malignant type (Morrison, 1998; Misdorp *et al.*, 1999).

A major problem is accurate prognosis for post-surgical mammary cancer cases. Prognosis is based on several tumour and host characteristics, histological type being the most important (Yamagami *et al.*, 1996; Fossum, 1997; Misdorp, 2002; Williams, 2003). Tumour grade and degree of invasion (stage) are also of prognostic significance (Gilbertson *et al.*, 1983; Misdorp *et al.*, 1999; Williams, 2003).

Systems proposed for histological grading of canine mammary carcinomas by Misdorp (2002) and Gilbertson *et al.* (1983) were based on a combination of cellular and predominantly nuclear features.

In human patients, the most common current method for histological grading of invasive breast carcinomas is the "Elston and Ellis method"

0021-9975/$ - see front matter
doi:10.1016/j.jcpa.2005.05.003

(Sloane *et al.*, 1999), which is the latest modification of the "Bloom and Richardson method". Grading by this method showed a strong relation to prognosis in a large number of patients followed-up for long periods (Elston and Ellis, 1991). In feline patients with mammary carcinomas, the same method was predictive of survival one year post-surgery (Castagnaro *et al.*, 1998). Recently, it has also been used in studies on canine mammary tumours (Karayannopoulou *et al.*, 2001; Nieto *et al.*, 2003; Reis *et al.*, 2003).

In the present study the prognostic validity of the Elston and Ellis method was examined in canine mammary carcinoma cases followed-up for 2 years.

Materials and Methods

Animals

Female dogs ($n=85$) with infiltrating mammary carcinomas (including carcinomas in benign tumours in which the malignant component predominated) were studied. The animals, aged 7–13 years (mean $\pm$ SD $= 9.5 \pm 1.8$), showed no clinical or radiological evidence of distant metastasis. They were selected from cases treated surgically between 1995 and 2000. The mammary tumours were excised by simple mastectomy or regional mastectomy (Misdorp, 2002), with or without the superficial inguinal lymph nodes. In cases of multiple tumours in a chain, the whole chain was resected. The animals were followed up every 4–6 months for at least 2 years after mastectomy. When the owner reported a problem, the dog was admitted to the clinic for physical and radiological examination to detect any local recurrence or distant metastasis. Animals that died from unrelated causes or were lost to follow-up during the 2-year period were excluded from the study. Euthanasia was performed only in the terminal stage of disease.

Clinical and Histopathological Evaluation

Tumour size. Mammary tumours were classified by size according to the World Health Organization Clinical Staging System TNM (Owen, 1980), as T_1 (<3 cm maximum diameter), T_2 (3–5 cm maximum diameter) and T_3 (>5 cm maximum diameter). In cases of multiple tumours, the largest one was used as the basis for classification.

Tumour type. Representative sections of each tumour (from the central core to periphery) and the excised lymph nodes were fixed in 10% buffered formalin, processed by routine methods, embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin (HE). Gomori's reticulin stain was employed for distinguishing spindle cell carcinomas from fibrosarcomas. Histopathological findings were recorded and used to classify the tumours according to the criteria of a recently validated system (Misdorp *et al.*, 1999). In cases with multiple tumours, the most malignant one as defined by Misdorp (2002) was recorded.

Tumour grade. Histological grading was performed on HE-stained sections. According to the Elston and Ellis (1991) method, the grade for each case was derived from an assessment of (1) tubule formation, (2) nuclear pleomorphism, and (3) mitotic counts, each feature being scored 1 to 3 points. The scores were then added to obtain the tumour grade, as follows: 3–5 points, well-differentiated carcinoma (grade I); 6–7 points, moderately differentiated carcinoma (grade II); 8–9 points, poorly differentiated carcinoma (grade III). Grading was carried out by one veterinary pathologist and, without prior knowledge of the results, confirmed by a second pathologist.

Mitotic counts were made with a Zeiss Axiolab microscope and $\times 40$ objective (field diameter, 0.575 mm; field area, 0.260 mm^2) in a manner that ensured equivalence with assessments made by Elston and Ellis (1991). Up to 8 mitoses per10 fields scored 1 point, 9–18 mitoses 2 points, and more than 19 or more mitoses 3 points.

Statistics

The predictive value of histological grade in terms of biological behaviour was evaluated not only in the total of the 85 cases of mammary carcinoma, but separately in the cases of simple carcinoma, the most common and malignant type. Sometimes, because of the small number of dogs, grade I and II carcinomas were grouped as "differentiated" for comparison with grade III (undifferentiated). The likelihood that increased histological grade of malignancy was associated with an increased risk of death within 2 years after mastectomy was determined with the Chi-square test for fourfold tables. Odds ratio (OR) and 95% confidence limits (CL) of OR were also computed. The possible relationship between frequency of deaths during the follow-up period and the age of the dogs at mastectomy was explored by means of a non-parametric test (Mann-Whitney) for two independent samples, while Chi-square test (or Fisher exact method) was used to determine the correlation between histological type, tumour size or lymph node status and number of deaths (Armitage

Table 1
Relationship between histological grading and tumour type in 85 dogs with mammary carcinoma

Histological type	*Number and percentage of cases*			*Total*
	Grade I	*Grade II*	*Grade III*	
Simple carcinoma	10 (18.2%)	17 (30.9%)	28 (50.9%)	55 (64.7%)
Complex carcinoma	7(87.5%)	1 (12.5%)	–	8 (9.4%)
Special type carcinoma	–	3 (75%)	1 (25%)	4 (4.7%)
Carcinoma in benign tumour	10 (55.6%)	7 (38.9%)	1 (5.6%)	18 (21.2%)
Total	27 (31.8%)	28 (32.9%)	30 (35.3%)	85 (100%)

and Berry, 1989), not only in the total number of cases but also in relation to histological grade. Survival curves obtained by the Kaplan-Meier method and the log rank test were used for further evaluating the correlation between histological grade or nodal metastasis and prognosis (Kaplan and Meier, 1958). For all statistical analyses, a P value of ≤ 0.05 was considered significant.

Results

Tumour Size and Histopathological Characteristics of the Tumours

According to their maximum diameter, the tumours were classified as T_1 in 9/85 (10.6%), T_2 in 23/85 (27.1%) and T_3 in 53/85 (62.3%) dogs.

The most frequently represented tumour type was simple carcinoma (SC) (55/85; 64.7%), followed by carcinoma in benign tumour (CBT) (18/85; 21.2%), complex carcinoma (CC) (8/85; 9.4%) and special type (spindle cell) carcinoma (STC) (4/85; 4.7%), as presented in Table 1. Excision of the superficial inguinal lymph nodes was performed in 66 dogs (46 with SC, 12 with CBT, 6 with CC and 2 with STC). Histopathological evaluation of these nodes revealed metastasis in 28 (42.4%) cases (26 with SC and 2 with STC).

The histological grades of the 85 cases were as follows: grade I, 27(31.8%); grade II, 28(32.9%); grade III, 30(35.3%). The relationship between tumour grading and histological type is presented in Table 1. Of the 66 dogs in which lymph node examination was performed, 23 had grade I tumours (10 SC, 7 CBT, 6 CC), 17 had grade II (12 SC, 4 CBT, 1 STC) and 26 had grade III (24 SC, 1 CBT, 1 STC); lymph node metastasis was found in 2 dogs with grade I tumours (SC), 10 with grade II (9 SC, 1 STC) and 16 with grade III (15 SC, 1 STC).

Case Outcome 2 Years after Mastectomy

At this time, 46/85 (54.1%) dogs were still alive with no evidence of recurrence or distant metastasis, while the remaining 39 (45.9%) animals had died or been humanely destroyed because of metastatic disease. A significant difference ($P<0.01$) in age between surviving (9 ± 1.7 years) and dead (10 ± 1.7 years) dogs was found. Of the 46 survivors, 18 had SC, 8 CC, 2 STC and 18 CBT; 7 of these tumours were T_1, 14 T_2 and 25 T_3. Of the 39 dead dogs, 37 had SC and 2 STC; 2 of these tumours were T_1, 9 T_2 and 28 T_3. Simple carcinoma was associated with a significantly ($P<0.001$) lower survival rate than that of the other types of carcinoma combined. No significant difference between tumour size and number of deaths was found. During the 2-year follow-up period, 24 of the 28(85.7%) dogs with lymph node metastasis (LN^+) died (22 with SC, 2 with STC), while of the 38 animals without nodal metastasis (LN^-) only 8 (21.1%) with SC died. A significant difference ($P<0.001$) between LN status and number of deaths was found.

The numbers of deaths within the 2-year follow-up period were 0/27 (0%) for dogs with grade I carcinoma, 13/28 (46.4%) for dogs with grade II carcinoma and 26/30 (86.7%) for dogs with grade III carcinoma. The frequency of deaths in relation to tumour grade and type of carcinoma is shown in Table 2. In the 26 dogs with simple carcinoma and lymph node metastasis, the frequency of deaths was

Table 2
Frequency of deaths by histological type and grade of tumour, in 85 dogs with mammary carcinoma, within 2 years after mastectomy

Histological type	*Number and percentage of deaths*			*Total*
	Grade I	*Grade II*	*Grade III*	
Simple carcinoma	0/10 (0%)	12/17 (70.6%)	25/28 (89.3%)	37/55 (67.3%)
Complex carcinoma	0/7 (0%)	0/1 (0%)	–	0/8 (0%)
Special type carcinoma	–	1/3 (33.3%)	1/1 (100%)	2/4 (50%)
Carcinoma in benign tumour	0/10 (0%)	0/7 (0%)	0/1 (0%)	0/18 (0%)
Total	0/27 (0%)	13/28 (46.4%)	26/30 (86.7%)	39/85 (45.9%)

0% (0/2 dogs) for grade I, 77.8% (7/9 dogs) for grade II, and 100% (15/15 dogs) for grade III tumours; both LN^+ dogs with special type (spindle cell) carcinoma (one of grade II and one of grade III) died within the follow-up period. As regards possible correlation between lymph node status and number of deaths during the 2-year period, all the dogs with grade I tumours (2 LN^+ and 21 LN^-) survived, while in dogs with grade II carcinoma no significant difference was found (LN^+: 2 alive, 8 dead; LN^-: 5 alive, 2 dead); however, in dogs with grade III tumours (LN^+: 0 alive, 16 dead; LN^-: 4 alive, 6 dead) the difference was significant ($P<0.05$; Fisher exact method). The mean age of the dogs with grade I tumours (all alive) was 8.4 ± 1.5 years; no significant age difference between alive (A) and dead (D) dogs was found either in grade II cases (A: 9.8 ± 1.9 years; D: 10.2 ± 1.6 years) or in grade III cases (A: 9.7 ± 1.3 years; D: 10 ± 1.8 years). The size of grade I tumours was T_1 in 5 cases, T_2 in 8, and T_3 in 14 (all alive); no significant difference between tumour size and number of deaths was found in grade II cases (A: $2T_1$, $4T_2$, $9T_3$, and D: $1T_1$, $1T_2$, $11T_3$) or in grade III cases (A: $2T_2$, $2T_3$, and D: $1T_1$, $8T_2$, $17T_3$).

Comparison between differentiated (grade I and II) and undifferentiated (grade III) carcinomas showed a 21-fold increased risk of death in the latter group ($P<0.001$; odds ratio(OR)=21; 95% confidence limits(CL)=5.5–87.8). Grade III carcinomas were associated with an approximately 7-fold increase in risk of death as compared with grade II carcinomas ($P<0.01$; OR=7.50; 95% CL=1.80–33.8). In cases of simple carcinoma, the percentage of deaths (89.3%) in dogs with undifferentiated tumours indicated a 10-fold greater risk than that in dogs with differentiated tumours ($P<0.001$; OR=10.42; 95% CL=2.19–56.55).

Survival was worse in dogs with grade III carcinomas than in those with grade II ($P<0.05$) or grade I ($P<0.001$) tumours (Fig. 1). In simple carcinoma cases, there was no significant difference ($P=0.878$) in survival between dogs with grade II and grade III tumours, both being associated with a poor prognosis (Fig. 2). However, comparison of differentiated (grade I and II) with undifferentiated (grade III) simple carcinomas indicated a significant ($P<0.05$) difference in survival (Fig. 3). Dogs with simple carcinoma and lymph node metastasis had a significantly worse survival rate ($P<0.001$) than did those without nodal metastasis (Fig. 4).

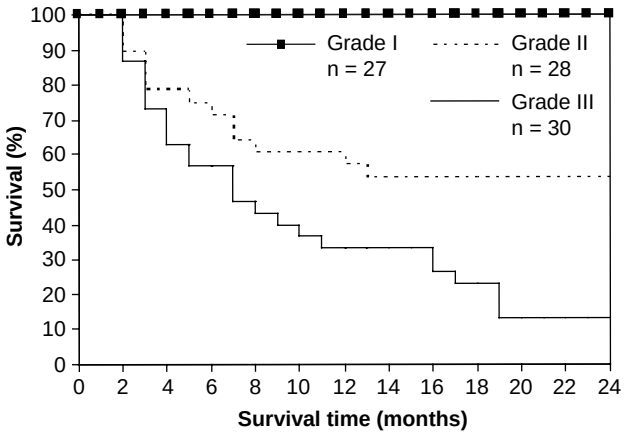

Fig. 1. Relationship between histological grade and 2-year post-surgical survival in 85 dogs with mammary carcinoma. Significance: grade I vs II, $P<0.001$; grade I vs III, $P<0.001$; grade II vs III, $P<0.05$.

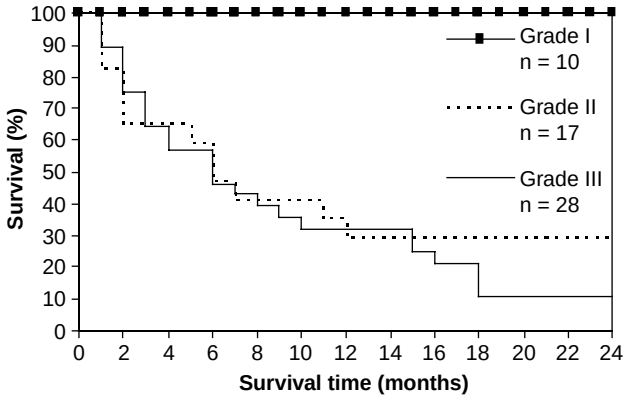

Fig. 2. Relationship between histological grade and 2-year post-surgical survival in 55 dogs with simple mammary carcinoma. Significance: grade I vs II, $P<0.05$; grade I vs III, $P<0.05$; grade II vs III, not significant.

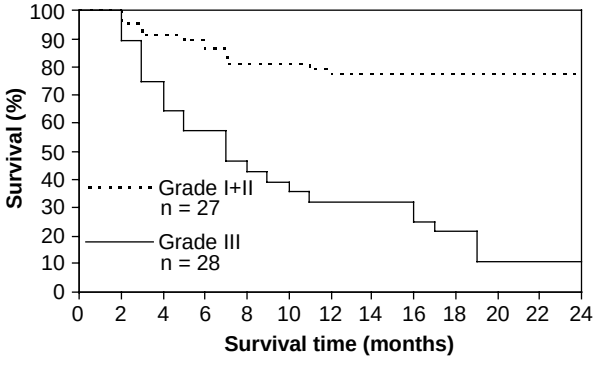

Fig. 3. Relationship between differentiated (grade I+II) or undifferentiated (grade III) tumours and 2-year post-surgical survival in 55 dogs with simple mammary carcinoma. Significance: grade I+II vs III, $P<0.05$.

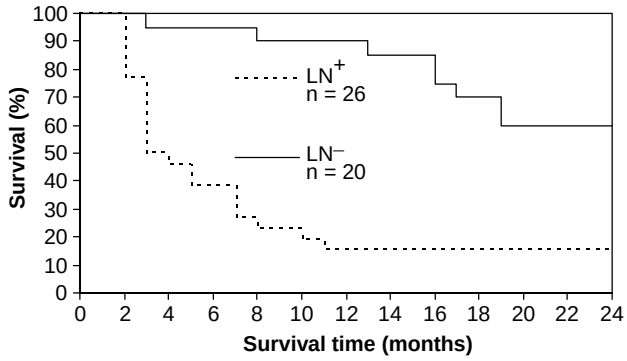

Fig. 4. Relationship between LN (lymph node) status and 2-year post-surgical survival in 46 dogs with simple carcinoma. Significance: LN^+ vs LN^-, $P<0.001$.

Discussion

Irrespective of the method used for tumour grading (histological or nuclear) such grading has always had an element of subjectivity, resulting in reduced reproducibility of results (Elston and Ellis, 1991; Castagnaro *et al.*, 1998; De Vico *et al.*, 2002). However, histological grading was found to be related to more objective prognostic indicators of tumour malignancy, such as S-phase fraction or cell proliferation index Ki-67 (Elston and Ellis, 1991; Pérez Alenza *et al.*, 1995; Nieto *et al.*, 2003). Additionally, the improved method of Elston and Ellis (1991) for human histological grading reduced problems of consistency and reproducibility to a minimum.

The measurement of only one of the parameters (tubule formation, nuclear pleomorphism, mitotic count) associated with histological grade is unlikely to provide powerful prognostic information (Baynes *et al.*, 1985; Elston and Ellis, 1991; Misdorp, 2002). Complete histological grading is therefore preferable to nuclear grading for accurate prognosis.

Survival time is considered a useful criterion for evaluating prognosis in both man and animals (Misdorp, 1987). In the present study, survival during a period of 2 years after surgical treatment was preferred to assessment of the disease-free interval, as the latter would have been subject to possibly unreliable observations by the owners. However, factors such as different types of therapy and deaths due to unrelated causes or euthanasia were considered by Gilbertson *et al.* (1983) to be against the use of survival time as an endpoint. To overcome these factors, every dog included in this study was treated by surgery alone, dogs that died from unrelated causes were excluded, and euthanasia when necessary was performed only in the terminal stage of disease.

In the present study, a correlation between histological type and grade was evident (Table 1). Carcinomas with a comparatively favourable prognosis, such as CBTs or even CCs (Benjamin *et al.*, 1999; Misdorp *et al.*, 1999), were usually of grade I or II. On the other hand, simple carcinomas (the most malignant type) were usually grade III or II. Similar observations were reported in human patients by Elston and Ellis (1991).

Because of the diversity of histological typing criteria, grading methods and endpoints used in different studies on the prognostic value of histological grading in canine mammary cancer, the results of such studies are difficult to compare (Brodey *et al.*, 1983; Gilbertson *et al.*, 1983; Benjamin *et al.*, 1999). In the only study (Misdorp and Hart, 1976) similar to ours, 50% of dogs with grade I mammary carcinomas and sarcomas, 64% with grade II, and 79% with grade III died within 2 years of surgical treatment. These results differed from our findings, possibly due to the use of a less refined grading method and the inclusion of sarcomas, which have the least favourable prognosis of all mammary tumours (Hellmén *et al.*, 1993; MacEwen and Withrow, 1996; Misdorp, 2002).

In human patients, a highly significant correlation between histological grade and prognosis was found by Elston and Ellis (1991); survival was worse in women with poorly differentiated tumours than in those with well-differentiated ones and patients with moderately differentiated tumours also had poor survival. In our study, similar results were found, especially in simple carcinoma cases (Figs 1 and 2), confirming the particularly poor prognosis of this histological type (Misdorp, 2002).

In dogs with mammary carcinomas, Gilbertson *et al.* (1983), by assessing nuclear grade, found that undifferentiated tumours were >22 times more likely than differentiated tumours to recur. The present study showed a 21-fold increased risk of death in dogs with undifferentiated carcinomas than in those with differentiated tumours.

The frequency of deaths differed not only according to histological grade but also according to tumour type (Table 2), which is a well-known prognostic factor in both human and canine patients (Fossum, 1997; Misdorp, 2002; Williams, 2003). CBTs (in which the malignant component had largely replaced the benign tumour) and CCs appeared to have a good prognosis, but the number of dogs examined was relatively small. Simple carcinomas, compared with all other carcinomas combined, had a particularly poor prognosis ($P<0.001$), as also reported by Misdorp

(2002). In dogs with high-grade simple carcinomas and metastasis to regional lymph nodes, the frequency of deaths was even worse. According to many veterinary authors, lymph node involvement was related to prognosis (Hellmén *et al.*, 1993; Yamagami *et al.*, 1996; Misdorp, 2002), as in human reports (Todd *et al.*, 1987; Elston and Ellis, 1991). Similarly, in the present study, lymph node metastasis worsened the prognosis; survival in dogs with LN^+ simple carcinomas was significantly worse ($P<0.001$) than in dogs without LN metastasis (Fig. 4).

In canine mammary cancer, the age of the dog at mastectomy is considered by some authors to be a factor of prognostic significance (Hellmén *et al.*, 1993; Misdorp *et al.*, 1999; Pérez Alenza *et al.*, 2000). In this study, although there was a significant difference in the mean age between surviving and dead dogs, no such difference existed when comparisons were made in relation to histological grade. Therefore, the predictive value of histological grade seemed not to be influenced by the age of the dog at the time of mastectomy. Tumour size is considered an important prognostic factor—small tumours having a better prognosis than large ones—in both canine (Yamagami *et al.*, 1996; Misdorp, 2002; Williams, 2003) and human patients (Haybittle *et al.*, 1982; Elston and Ellis, 1991). However, in the present study, no significant relation between tumour size and number of deaths was found, in agreement with some other reports (Else and Hannant, 1979; Hellmén *et al.*, 1993), especially when lymph node metastasis or vessel invasion was present (Kurzman and Gilbertson, 1986). It is likely, therefore, that tumour size did not influence the predictive value of histological grade in this study.

In conclusion, histological grading of canine mammary carcinomas by the Elston and Ellis method was significantly related to prognosis, especially in cases of simple carcinoma. Its routine use should be helpful in indicating appropriate post-surgical treatment.

References

Armitage, P. and Berry, G. (1989). *Statistical Methods in Medical Research* (2nd Edit.), Blackwell Scientific Publications, Oxford.

Baynes, S. C., Ellis, I. O., Mann, R., Lowe, J., Elston, C. W. and Blamey, R. W. (1985). Evaluation of nuclear morphometric measurements as prognostic factors in primary breast cancer. *Journal of Pathology*, **146,** 271A.

Benjamin, S. A., Lee, A. C. and Saunders, W. J. (1999). Classification and behaviour of canine mammary epithelial neoplasms based on life-span observations in Beagles. *Veterinary Pathology*, **36,** 423–436.

Brodey, R. S., Goldschmidt, M. H. and Roszel, J. R. (1983). Canine mammary gland neoplasms. *Journal of the American Animal Hospital Association*, **19,** 61–90.

Castagnaro, M., Casalone, C., Bozzetta, E., De Maria, R., Biolatti, B. and Caramelli, M. (1998). Tumour grading and the one-year post-surgical prognosis in feline mammary carcinomas. *Journal of Comparative Pathology*, **119,** 263–275.

Davidson, E. B. (2003). Treatment of mammary tumors in dogs and cats. *Proceedings of the 2003 North American Veterinary Conference, Orlando, Florida* 2003, pp. 1036–1038.

De Vico, G., Sfacteria, A., Maiolino, P. and Mazzullo, G. (2002). Comparison of nuclear morphometric parameters in cytologic smears and histologic sections of spontaneous canine tumors. *Veterinary Clinical Pathology*, **31,** 16–18.

Else, R. W. and Hannant, D. (1979). Some epidemiological aspects of mammary neoplasia in the bitch. *Veterinary Record*, **104,** 296–304.

Elston, C. W. and 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,** 403–410.

Fossum, T. W. (1997). *Small Animal Surgery*, Mosby-Year Book, St Louis, Missouri, pp. 539–544.

Gilbertson, S. R., Kurzman, I. D., Zachrau, R. E., Hurvitz, A. I. and Black, M. M. (1983). Canine mammary epithelial neoplasms: biologic implications of morphologic characteristics assessed in 232 dogs. *Veterinary Pathology*, **20,** 127–142.

Haybittle, J. L., Blamey, R. W., Elston, C. W., Johnson, J., Doyle, P. J., Campbell, F. C., Nicholson, R. I. and Griffiths, K. (1982). A prognostic index in primary breast cancer. *British Journal of Cancer*, **45,** 361–366.

Hellmén, E., Bergström, R., Holmberg, L., Spangberg, I. B., Hansson, K. and Lindgren, A. (1993). Prognostic factors in canine mammary tumors: A multivariate study of 202 consecutive cases. *Veterinary Pathology*, **30,** 20–27.

Kaplan, E. L. and Meier, P. (1958). Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association*, **53,** 457–481.

Karayannopoulou, M., Kaldrymidou, E., Constantinidis, T. C. and Dessiris, A. (2001). Adjuvant post-operative chemotherapy in bitches with mammary cancer. *Journal of Veterinary Medicine (A)*, **48,** 85–96.

Kitchell, B. E. and Loar, A. S. (1997). Diseases of the mammary glands. In: *Handbook of Small Animal Practice*, 3rd Edit., R. V. Morgan, Ed., W.B. Saunders, Philadelphia, pp. 615–625.

Kurzman, I. D. and Gilbertson, S. R. (1986). Prognostic factors in canine mammary tumors. *Seminars in Veterinary Medicine and Surgery (Small Animal)*, **1,** 25–32.

MacEwen, E. G. and Withrow, S. J. (1996). Tumors of the mammary gland. In: *Small Animal Clinical Oncology*, 2nd Edit., S. J. Withrow and E. G. MacEwen, Eds, W.B. Saunders, Philadelphia, pp. 356–372.

Misdorp, W. (1987). The impact of pathology on the study and treatment of cancer. In: *Veterinary Cancer Medicine*, G. H. Theilen and B. R. Madewell, Eds, Lea and Febiger, Philadelphia, pp. 53–70.

Misdorp, W. (2002). Tumors of the mammary gland. In: *Tumours in Domestic Animals*, 4th Edit., D. J. Meuten, Ed., Iowa State Press, Iowa, pp. 575–606.

Misdorp, W., Else, R. W., Hellmén, E. and Lipscomb, T. P. (1999). Histological Classification of Mammary Tumors of the Dog and the Cat. Armed Forces Institute of Pathology and the American Registry of Pathology and The World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology, Washington, D.C., USA, vol. 7, pp. 11–29.

Misdorp, W. and Hart, A. A. M. (1976). Prognostic factors in canine mammary cancer. *Journal of the National Cancer Institute*, **56,** 779–784.

Morrison, W. B. (1998). Canine and feline mammary tumors. In: *Cancer in Dogs and Cats: Medical and Surgical Management*, W. B. Morrison, Ed., Williams and Wilkins, Baltimore, Maryland, pp. 591–598.

Nieto, A., Pérez Alenza, M. D., Del Castillo, N., Tabanera, E., Castaño, M. and Peña, L. (2003). BRCA1 expression in canine mammary dysplasias and tumours: relationship with prognostic variables. *Journal of Comparative Pathology*, **128,** 260–268.

Owen, L. N. (1980). *The TNM Classification of Tumors in Domestic Animals,* 1st Edit., World Health Organization, Geneva, pp. 20–80.

Pérez Alenza, M. D., Peña, L., Del Castillo, N. and Nieto, A. I. (2000). Factors influencing the incidence and prognosis of canine mammary tumours. *Journal of Small Animal Practice*, **41,** 287–291.

Pérez Alenza, M. D., Rutteman, G. R., Kuipers-Dijkshoorn, N. J., Peña, L., Montoya, A., Misdorp, W. and Cornelisse, C. J. (1995). DNA flow cytometry of canine mammary tumours: the relationship of DNA ploidy and S-phase fraction to clinical and histological features. *Research in Veterinary Science*, **58,** 238–243.

Reis, A. L., Carvalheira, J., Schmitt, F. C. and Gärtner, F. (2003). Immunohistochemical study of the expression of E-cadherin in canine mammary tumours. *Veterinary Record*, **152,** 621–624.

Sloane, J. P., Amendoeira, I., Apostolikas, N., Bellocq, J. P., Bianchi, S., Boecker, W., Bussolati, G., Coleman, D., Connolly, C. E., Eusebi, V., De Miguel, C., Dervan, P., Drijkoningen, R., Elston, C. W., Faverly, D., Gad, A., Jacquemier, J., Lacerda, M., Martinez-Penuela, J., Munt, C., Peterse, J. L., Rank, F., Sylvan, M., Tsakraklides, V. and Zafrani, B. (1999). Consistency achieved by 23 European pathologists from 12 countries in diagnosing breast disease and reporting prognostic features of carcinomas. *Virchows Archiv*, **434,** 3–10.

Todd, J. H., Dowle, C., Williams, M. R., Elston, C. W., Ellis, I. O., Hinton, C. P., Blamey, R. W. and Haybittle, J. L. (1987). Confirmation of a prognostic index in primary breast cancer. *British Journal of Cancer*, **56,** 489–492.

Williams, L. (2003). Predictors of tumor response. *Proceedings of the North American Veterinary Conference, Orlando, Florida,* pp. 679–681.

Yamagami, T., Kobayashi, T., Takahashi, K. and Sugiyama, M. (1996). Prognosis for canine malignant mammary tumors based on TNM and histologic classification. *Journal of Veterinary Medical Science*, **58,** 1079–1083.

[*Received*, *June 21st*, *2004*
Accepted, *May 21st*, *2005*]