Multiple mammary tumours in a bitch: analysis of mitotic index, AgNOR count and c-erbB2 expression status: a case report

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Abstract: This case report of multiple mammary tumours in a Spitz bitch revealed the presence of three tumour masses, T-1, T-2 and T-3, of variable size, shape and texture on different mammary glands. Histopathological examination revealed these tumours to be of different histological type (T-1 – fibrosarcoma, T-2 – malignant mixed mammary tumour and T-3 – papillary adenocarcinoma). The mitotic index and AgNOR count was high in all three tumours indicative of a higher proliferation status of neoplastic cells while T-2 and T-3 showed overexpression of the c-erbB2 oncoprotein.

Keywords: multiple mammary tumours; bitch; c-erbB2; AgNOR

Mammary gland tumours are the most common type of tumours encountered in bitches followed by skin tumours (Neruker et al., 1989; Benjamin et al., 1999) and their incidence varies from 198 to 622.6 cases per 100 000 dogs per year (Bostock, 1986; Vail and MacEwen, 2000; Goldschmidt et al., 2001). Clinically, canine mammary tumours (CMT) occur either as single or multiple nodules, and, if multiple, can be of the same or different histological types. Grossly they vary from well-circumscribed nodules with stationary growth to large and sometimes ulcerated nodules, which grow rapidly and become fixed to adjacent tissues. The initial diagnosis of CMTs is based on clinical signs and symptoms and while fine-needle aspiration may be useful (Hellmen and Lindgren, 1989), histology is necessary for an accurate diagnosis (Allen et al., 1986). Malignant canine mammary tumours are more frequent than benign ones (Brodey et al., 1983; Shekhar et al., 2001; Reddy et al., 2009). Mammary tumours can metastasize usually to axillary and superficial inguinal lymph nodes (Fidler and Brodey, 1967; Hellmen et al., 1993) but may spread to other organs like lungs, liver, kidneys, skeletal muscles, spleen etc.

Despite major advances in diagnostics and therapeutics, the survival of bitches with mammary tumours has not been substantially improved. Biological parameters such as tumour cell proliferation, different hormonal receptors and other molecular biomarkers can act as important indicators for their timely diagnosis as well as prognosis. The cell proliferation status of tumours is assessed commonly by mitotic index, levels of argyrophilic nucleolar organizer regions (AgNORs), thymidine-labeling index, Ki-67 and levels of proliferating cell nuclear antigen (PCNA; Bedrossian, 1993). Three well characterized biomarkers, i.e., the c-erbB2 oncogene (Tsutsui et al., 2002), estrogen receptors and progesterone receptors have been used in the clinical analysis of breast cancer in humans by immunohistochemistry and fluorescence *in situ* hybridization (FISH). Overexpression of the c-erbB2 oncoprotein has been reported in human breast and ovarian cancers and has been shown to be related to the clinical prognosis, relapse-free survival time, oestrogen or progesterone hormonal status, metastasis and chemotherapy response in mammary adenocarcinomas.

Case description

An 11-year old Spitz bitch, weighing 8.5 kg presented to the Veterinary Clinic, Indian Veterinary

Research Institute, Izatnagar with multiple growths on different mammary glands which had proceeded for five to six months. The animal had not eaten for two days and was very weak, anaemic and inactive. A blood sample was collected for complete blood count (CBC) and revealed a Hb of 6.2 gm%, PCV 21%, TEC 5.8 millions/cu/mm, TLC 8 × 10^3/cu/mm and DLC – neutrophils 65%, eosinophils 3%, basophils 0%, lymphocytes 27% and monocytes 5%. The animal was given symptomatic treatment on that day but died that night and was sent for post mortem analysis.

At post mortem examination the animal was examined thoroughly and was found to have three growths of variable sizes on the ventral side of the abdomen comprising different mammary glands. The first growth (T-1) was very large (15 × 10 cm^2), firm and weighed around 1100 gms. It involved the 1st and 2nd mammary glands on the left side. The second growth (T-2) was present on the 2nd mammary gland on the right side and had given rise to an ulcerated wound accompanied by suppuration. This growth was hard, firm and flat in appearance and had a size of 6 × 3 cm^2. The third growth (T-3) was present on the 4th and 5th mammary glands on the right side. This growth was firm and was 3 × 2 cm^2 in size of. Further examination of the carcass revealed moderate congestion of lungs and intestine; liver and kidneys were pale while the other organs showed no unusual signs.

Representative samples of tissues from these tumours and other organs (lung, liver, kidney and supramammary lymph nodes) were collected in 10% buffered formalin for histopathological examination, AgNOR counting and immunohistochemical staining for c-erbB2 oncoprotein. A tissue section of 5µm thickness was prepared and stained by hematoxylin and eosin using standard procedures for histopathological examination. The tissue section of T-1 revealed densely packed fibroblasts with plump round or oval, sometimes fusiform nuclei with one or more prominent nucleoli with moderate amount of collagen deposition. It was classified as a fibrosarcoma of the mammary gland (Figure 1). Acini and ducts were compressed, distorted and appeared atrophied. Necrosis was evident in two cases accompanied by infiltration of mononuclear cells and neutrophils.

The T-2 tumour revealed proliferation of the epithelium of the acini arranged in multiple layers which obliterated the lumen partially or completely and in some areas had papillary projections (Figure 2 and 5). Neoplastic cells had vesicular large hyperchromatic nuclei and scanty cytoplasm. In other areas, there was extensive myoepithelial proliferation, which frequently transformed into chondroid and cartilaginous tissue replacing the mammary gland parenchyma. Areas showing higher proportion of epithelial tissue and carcinomatous changes showed moderate numbers of mitotic figures. This was diagnosed as a malignant mixed mammary tumour. In several cases, areas of necrosis were evident with infiltration of neutrophils, lymphocytes and macrophages.

The T-3 tumour was diagnosed as a papillary adenocarcinoma and showed extensive proliferation of the lining epithelial cells of the acini as well as mammary gland ducts resulting in papillary projec-

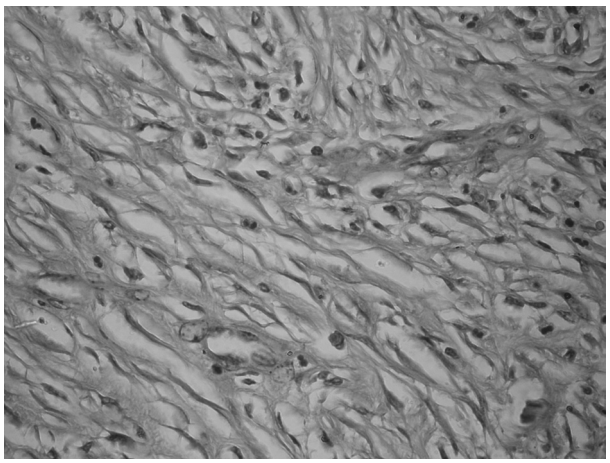

Figure 1. Fibrosarcoma. Fusiform cells proliferating in a haphazard pattern showing hyperchromatic nuclei. H&E, 250×

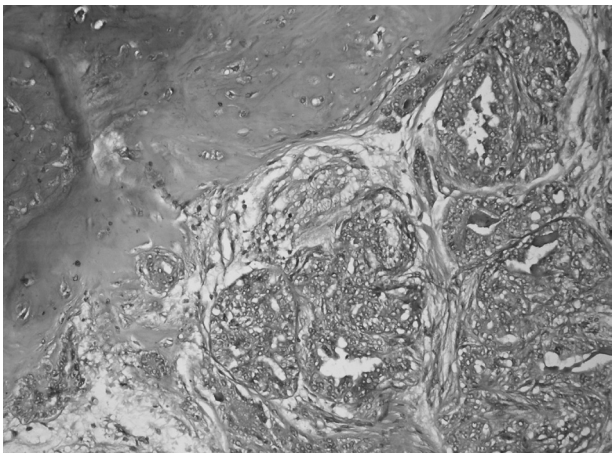

Figure 2. Malignant mixed mammary tumour. Tumour tissue showing epithelial and myoepithelial components with transformation into chondroid tissue. H&E, 100×

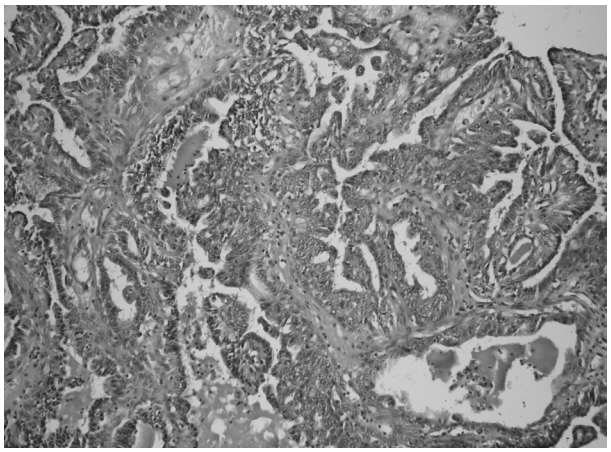

Figure 3. Papillary adenocarcinoma. Proliferating neoplastic epithelial cells in the form of papillary projections and presence of mitotic figures. H&E, 100×

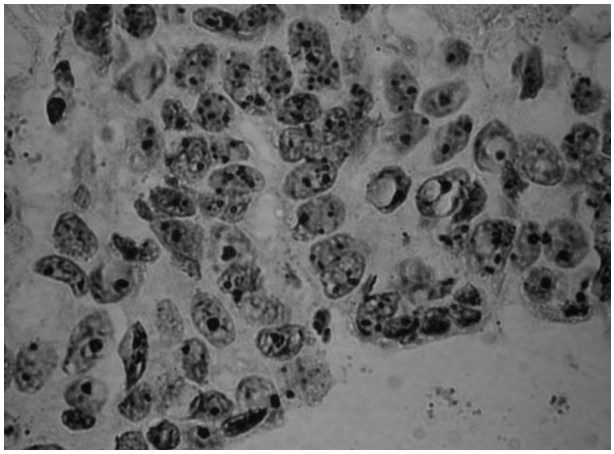

Figure 4. Papillary adenocarcinoma. Presence of multiple small to medium sized AgNOR spots in the nuclei of tumour cells. AgNOR, 400×

tions into the lumen and was attached with a thin connective tissue stalk and stroma (Figures 3 and 4). The neoplastic cells of columnar to cuboidal shape had medium to large sized, round or oval vesicular nuclei with scanty cytoplasm.

In comparison, tissue sections from other organs without metastatic cells in their parenchyma (kidney) showed few areas of necrosis.

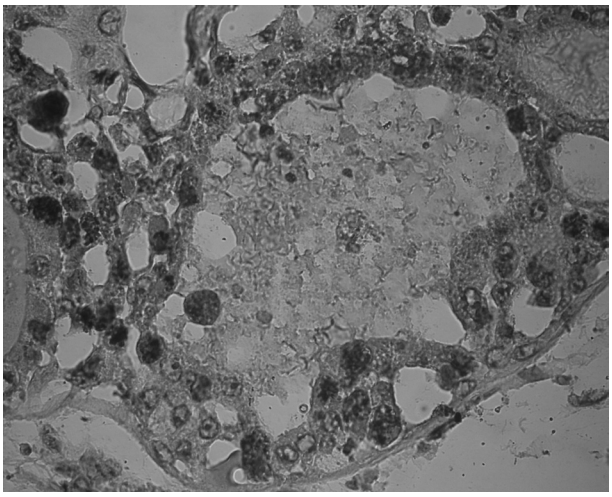

Figure 5. Malignant mixed mammary tumour. Strongly immunostained epithelial cell positive for c-erbB2 oncoprotein. IP×AEC, 400×

The mitotic index and AgNOR count index was determined following the methods described by Yu et al. (1992) and Crocker (1989), respectively. Mitotic figures and AgNOR counts had higher values in the papillary adenocarcinoma and malignant mixed mammary tumour than in the fibrosarcoma (Table 1).

Immunohistochemical staining for the c-erbB2 oncoprotein was carried out following a standard procedure using mouse monoclonal anti-c-erbB2 antibody (Sigma-E 2777). Immunostaining revealed strong immunolabelling in the membrane and cytoplasm of neoplastic cells of the papillary adenocarcinoma (T-3), epithelial component of the malignant mixed mammary tumour (T-2) while the fibrosarcoma (T-1) was negative for immunostaining.

DISCUSSION AND CONCLUSIONS

Cases of multiple canine mammary tumours of different histological types are very rare. The histological types found in this case were also reported by other researchers (Misdorp et al., 1999; Funakoshi et al., 2000; Reddy et al., 2009) but mostly in solitary cases. The mitotic index revealed that the tumour mass had

Table 1. Mitotic index and AgNOR count of T-1, T-2 and T-3

S. No.	Tumour	Histological type	Mitotic index	AgNOR Count
1	T-1	fibrosarcoma	1.7	3.10
2	T-2	malignant mixed	2.04	3.46
3	T-3	papillary adenocarcinoma	2.18	3.24

a good proportion of cells in the M-phase of the cell cycle. The AgNOR count has been associated with the degree of cell proliferation (Crocker et al., 1989; Hall and Levison, 1990; Trere, 2000) and in canine mammary tumours it found to be higher in malignant tumours than benign ones (Bostock et al., 1992; Kumar et al., 2010). The AgNOR counts found in T-1, T-2 and T-3 were elevated and indicate that cells were in an active mitotic stage.

Functional abnormality of the c-erbB2 oncogene is believed to be a critical event in the carcinogenesis of glandular tissues, especially of the breast where overexpression of c-erbB2 oncoprotein has been frequently associated with the malignancy and poor prognosis of breast cancers (Yokota et al. 1986; Bruman et al., 1990). In the present case the tumours T-2 and T-3 were positive for c-erbB2 overexpression while T-1 was negative. Both T-2 and T-3 consisted of neoplastic cells of epithelial origin and it has been reported that c-erbB2 overexpression is mostly restricted to mammary tumours of epithelial origin (Yakota et al., 1986; Rungsipipat et al., 1999; Kumar et al., 2009). Overexpression of c-erbB2 has been correlated with poor prognosis of human breast cancers by many researchers (Yakota et al., 1986; Bruman et al., 1990). In this case the animal died and T-2 and T-3 were found to have overexpression of c-erbB2. Thus, there was the possibility that if biopsies of these tumours had been carried out while the animal was still alive then the poor prognosis of the case could have been made and appropriate emergency treatment could have been administered. Therefore, before designing any treatment for canine mammary tumours the expression level of c-erbB2 along with other parameters should be considered so as to improve prognosis.

Acknowledgements

The authors are thankful to the Director of the Institute and Head of the Division of Pathology for providing facilities in which to carry out the work. P.K. is grateful to the ICAR for providing JRF during the course of the study.

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Received: 2010–07–24
Accepted after corrections: 2010–11–21

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