Bulgarian Journal of Veterinary Medicine (2006), **9**, No 3, 211–219

STUDY ON THE CORRELATION BETWEEN THE CYTOLOGICAL AND HISTOLOGICAL TESTS IN THE DIAGNOSTICS OF CANINE SPONTANEOUS MAMMARY NEOPLASMS

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Summary

Simeonov, R. & D. Stoikov, 2006. Study on the correlation between the cytological and histological tests in the diagnostics of canine spontaneous mammary neoplasms. *Bulg. J. Vet. Med.*, **9**, No 3, 211–219.

In the current study, the correlation between cytological and histological diagnoses in the study of 70 cases of spontaneous canine mammary tumours was investigated. This comparison was performed only with regard to the malignancy and the type of the neoplastic formations. Of all tested tumours, a correct cytological diagnosis was established for 57 of them (81.4%), while an incorrect diagnosis was established for 13 (18.6%). Of all tested tumours, there were 5 (7.1%) false positive results, and 8 (11.4%) false negative results. From a total of 13 benign tumours, the cytological diagnosis was correct for 11 tumours (84.6%). There were 2 false positive diagnoses among them, which constituted 15.4% of all benign tumours. From a total of 57 malignant tumours, the diagnosis was correct for 46 of them (80.7%). There were 3 false positive and 8 false negative diagnoses, which constituted 5.3% and 14.0% of the total number, respectively.

Key words: cytological diagnostics, dogs, mammary gland, tumours

INTRODUCTION

Mammary gland neoplasms are the most commonly seen tumours in intact female dogs (Bostock, 1986; MacEwen & Withrow, 1996; MacEwen & Withrow 2001; Moe, 2001; Morris & Dobson, 2001; Misdorp, 2002; Sorenmo, 2003). According to Brodey *et al.* (1983) and Yager *et al.* (1993) about 95% of them are of epithelial origin, while the other 5% are mesenchymal. Based on histological criteria, it was determined that half of the surgically removed mammary neoplasms in bitches were malignant (Fidler *et al.*, 1967; Bostock, 1977; Priester, 1979; Brodey *et al.*, 1983; Sorenmo, 2003).

The global tendency is to look for fast and inexpensive methods for tumour diagnostics. The routinely used histological test is relatively slow, and it requires invasive sampling of material for studying (biopsy), fixation, and additional processing. It is important to establish quickly a diagnosis, especially during ongoing surgery, so that the appropriate type of surgical intervention is chosen immediately. One of the methods for quick diagnostics of tumours is the cytological test. This diagnostic method has multiple significances. During the initial stage, it can be assessed whether the case is an inflammatory process or a neoplasm. If the diagnosis of a tumour growth is confirmed, a cytological test of the lymph nodes could provide information on the stage, progno-

sis, and the presence of metastases (Alleman & Bain, 2000; Baker & Lumsden, 2000). In the cases of apparently inoperable malignant neoplasms, the positive cytological diagnosis would make any confirming biopsy unnecessary (Allen *et al.*, 1986; Allen & Prasse, 1986; Zahariev, 1995; Alleman & Bain, 2000; Allison *et al.*, 2003). In cases of post-operative recurrence, the consequent cytological test could quickly and precisely indicate the need for further interventions. In most cases, a diagnosis can be established within a few minutes, and the sampling of material is not traumatic for the patient (Baker & Lumsden, 2000; Allison *et al.*, 2003; Simeonov, 2004; Simeonova *et al.*, 2004).

The correlation between cytological and histological testing in the diagnostics of spontaneous cases of mammary neoplasms in female dogs has been studied by a very small number of researchers. The results show 79% correlation of the cytologically established diagnoses to the ultimate histological tests (Hellmen & Lindgren, 1989; Zuccari *et al.*, 2001), and according to other studies – 63% or less (Menard *et al.*, 1986).

The scarce and somewhat contradictory information on the diagnostical value of cytological tests in the diagnostics of canine mammary neoplasms gave us a good reason to investigate the significance of this method of examination.

MATERIALS AND METHODS

The current research was performed on 70 cases of spontaneous mammary gland tumours in female dogs accepted for examination and treatment in the Clinic of Surgery and the Clinic of Obstetrics at the Faculty of Veterinary Medicine, Trakia University – Stara Zagora, as well as from patients of private veterinary dispensaries in Southeast Bulgaria, for the period 2002 – 2004. After surgical interventions, all mammary tumours were classified in accordance with the WHO Histological Classification of Mammary Tumours of the Dog and Cat (Misdorp *et al.*, 2001). The type distribution of the neoplastic formation was the following: fibroadenoma – n=9, mixed-type mammary benign tumour – n=4, tubulopapillary carcinoma – n=17, solid carcinoma – n=10, anaplastic carcinoma – n=7, fibrosarcoma – n=9, osteosarcoma – n=4, liposarcoma – n=6, and mixed-type malignant mammary tumour – n=4 – Table 1.

Preoperatively, specimens for cytological study were obtained from each tumour formation by fine needle aspiration biopsy (FNAB); after surgical removal of the formations, material for cytological study was obtained through imprint samples, smears, and scratching of cellular material from a freshly cut surface.

Table 1. Distribution of mammary gland specimens included in the study

Tumours	n
Fibroadenoma	9
Benign mixed mammary tumors	4
Tubulopapillary carcinoma	17
Solid carcinoma	10
Anaplastic carcinoma	7
Fibrosarcoma	9
Osteosarcoma	4
Liposarcoma	6
Carcinosarcoma	4

Syringes of 10 cm^3 and 22 G needles were used to perform the FNAB. The technique that was used for this manipulation was the following: the tumour forma-

tion was fixed with the thumb and the other fingers of the left hand, while the other hand inserted the needle with the attached syringe. Following this, negative pressure was applied, as the syringe's piston was quickly pulled. Maintaining the vacuum this way, the needle and the syringe were rapidly moved back and forth in different directions. Before the needle was pulled out, the piston was returned to its initial position, so that the pressure would be released. Having removed the needle, it was immediately detached from the syringe, and 2–3 cm^3 of air were sucked into it, so that it could be used to push the cells from the needle's lumen on a previously cleaned glass slide. In all cases, we tried to avoid the neoplasms' centres, because of the danger of aspirating necrotic matter. In the cases when the collected material was insufficient, we performed the manipulation on several different areas of neoplastic formations. Thus, the possibility for obtaining poor quality material (hemorrhagic, necrotic), or no material at all was minimized. The imprint samples were prepared by pressing the dried out cut surface of the material to the glass slide. When the material was semi-liquid in consistency, we prepared cytological smears. Thus, parts of the tumour formation were put on a slide, approximately one centimeter away from its edge. A second slide was placed at right angle to the first so that it lied almost flat (at 10-15 ^{o}) over the sample, and was moved quickly on first slide's surface in order to spread the material in a thin layer, which would provide better visualization of the finding.

To obtain cellular material from a freshly cut surface, we used a lancet blade to scrub a thin layer off the tumour's surface. After careful spreading of the cellular material on the slide, the samples were fixed at normal room temperature, for no less than 10 minutes, and were stained with Hemacolor® (Merck®, Darmstadt, Germany). This colouring method was chosen because of its simplicity and speed, combined with the option to obtain an excellent cellular information and the low cost of the method.

Mammary neoplasms were cytologically classified as benign or malignant according to the following criteria of malignancy by Tyler *et al.* (1999):

- anisocytosis: variations in the cells' sizes;
- pleomorphism: variations in the shapes of cells of the same type;
- hypercellularity: increase in the cell exfoliation because of weakened connections between cells;
- macrokaryosis: increase in nuclei's sizes;
- anisokaryosis: variations in the nuclei's sizes;
- multinucleation: increase in the number of nuclei;
- increased nuclear/cytoplasm ratio;
- nuclear molding: deformation of the nuclei;
- increased mitotic figures;
- abnormal mitoses;
- coarse chromatin pattern: coarse placement of nuclear chromatin;
- macronucleoli: increase in the sizes of the nucleoli;
- angular nucleoli: presence of angular nucleoli;
- anisonucleosis: changes in the sizes of nucleoli.

If three or more of the above tumour malignancy criteria were present, the tumour formations were cytologically classified as malignant, while all other cases were classified as benign. All cytologically established diagnoses were compared to the results of the ultimate histo-

logical tests. A parallel comparison between the cytological and histological diagnoses was performed with regard to the malignancy and the type of the neoplastic formations.

RESULTS

Of all 70 tested spontaneous neoplasias, cytological diagnosis on benign/malignant type of tumour was established correctly for 57 tumour formations (81.4%), while the other 13 (18.6%) tested neoplasias were diagnosed incorrectly – Table 2.

Of all tested tumours, there were 5 false positive results (7.1%) and 8 (11.4%) false negative results.

From a total of 13 benign tumours, cytological diagnosis was correctly established for 11 of them (84.6%). The correlation between cytological and histological diagnoses for benign mammary tumours was the following: fibroadenoma (Fig. 1 and Fig. 2) – 8 correct out of 9 (88.9%) (1 false positive diagnosis), and mixed-type benign mammary tumour – 3 correct out of 4 (75%) (1 false positive diagnosis). False positive diagnoses constituted 15.4% of all benign mammary neoplasia.

From a total of 57 malignant tumours, correct diagnosis was established for 46 of them (80.7%). The correlation between cytological and histological diagnoses for

Table 2. Correlation between the cytological and histological diagnoses with regard to the malignancy (benign or malignant) of studied neoplastic formations

Tumours	n	False-positive		False-negative		Correlation (%*)
		n	%	n	%	
I. Benign tumours						
Fibroadenoma	9	1	7.2	–	–	88.9
Benign mixed mammary tumours	4	1	7.2	–	–	75.0
Total benign	13	2	15.4	–	–	84.6
II. Malignant tumours						
Tubulopapillary carcinoma	17	–	–	3	5.3	82.4
Solid carcinoma	10	–	–	2	3.5	80.0
Anaplastic carcinoma	7	–	–	–	–	100.0
Fibrosarcoma	9	1	1.8	1	1.8	77.8
Osteosarcoma	4	–	–	–	–	100.0
Liposarcoma	6	–	–	1	1.8	83.3
Carcinosarcoma	4	2	3.5	1	1.8	25.0
Total malignant	57	3	5.3	8	14.0	80.7
Total	70	5	7.1	8	11.4	81.4

* percentage from positive histological diagnoses.

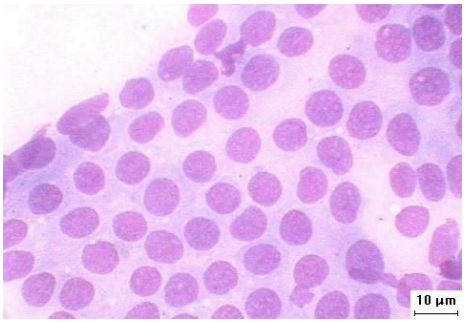

Fig. 1. Fibroadenoma. Fine-needle aspiration biopsy. Hemacolor staining.

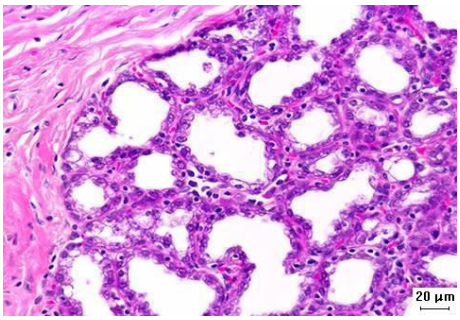

Fig. 2. Fibroadenoma. Acinous structures. Hematoxyllin/eosin staining (H/E).

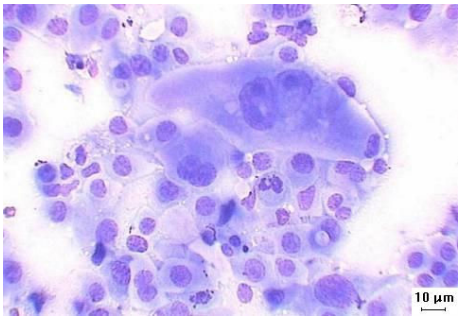

Fig. 3. Anaplastic carcinoma. Macrokaryosis, anisokaryois, nuclear malformation, coarse chromatin pattern. Fine-needle aspiration biopsy / Hemacolor staining.

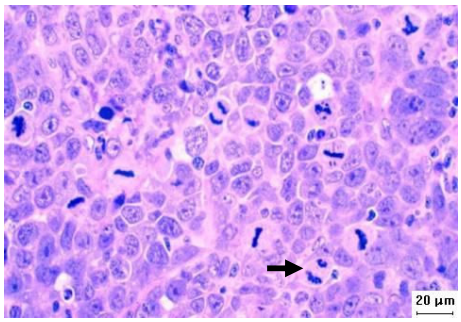

Fig. 4. Anaplastic carcinoma. Mitosis (arrow).

malignant mammary tumours was the following: tubulopapillary carcinoma – 14 correct out of 17 (82.4%) (3 false negative diagnoses), solid carcinoma – 8 correct out of 10 (80%) (2 false negative diagnoses), anaplastic carcinoma (Fig. 3, Fig. 4) – 7 correct out of 7 (100%), fibrosarcoma – 7 correct out of 9 (77.8%) (1 false positive and 1 false negative diagnosis), osteosarcoma – 4 correct out of 4 (100%), liposarcoma – 5 correct out of 6 (83.3%) (1 false negative diagnosis), and carcinosarcoma – 1 correct out of 4 (25%) (2 false positive and 1 false negative diagnoses). False positive and false negative diagnoses constituted 5.3% and 14.0% of the total number of malignant tumours, respectively.

DISCUSSION

The correlation between cytological and histological diagnoses in mammary neoplasia diagnostics is different in human and veterinary medicine. While the share of correct cytological diagnoses in human

medicine is above 90% (Abu-Salem, 2002; Ariga *et al.*, 2002; Chaiwun *et al.*, 2002; deGuzman *et al.*, 2002; Yu *et al.*, 2002; Berner *et al.*, 2003; Choi *et al.*, 2004), it is quite lower in veterinary medicine (Allen *et al.*, 1986; Helmen & Lindgren, 1989; Menard *et al.*, 1986; Siegl, 1999; Zuccari *et al.*, 2001). Our study was primarily related to the practical need of differentiation of malignancy of mammary neoplasia, that occurs during a surgical interventions. During the research, the greatest difficulties were encountered with mixed-type tumours and mesenchymal mammary tumours. According to us, this was due to the harder exfoliation of the cells while obtaining material for cytological testing. This opinion is supported by other authors as well (Allen *et al.*, 1986; Menard *et al.*, 1986; Helmen & Lindgren, 1989; Zuccari *et al.*, 2001). We think that, for these tumours, scrubbing cellular material off a freshly cut surface is more efficient than FNAB or the preparation of imprint samples, since scrubbing facilitates the obtaining of more cellular material. The higher correlation between cytologically and histologically established diagnoses in our study, in comparison with the research by Allen *et al.* (1986), Menard *et al.* (1986), Helmen & Lindgren (1989), Siegl (1999), and Zuccari *et al.* (2001), could be explained with the obtaining of cellular material by means of FNAB, combined with the simultaneous preparation of smears, imprint samples, and the scrubbing of material off a freshly cut surface.

The results from our research showed a higher percentage of false positive and false negative diagnoses than the results of other authors (Menard *et al.*, 1986; Helmen & Lindgren, 1989; Siegl, 1999; Zuccari *et al.*, 2001). False positive results are accepted to be the pathologist's mistake, commonly related to the lack of necessary experience, inadvertence, or usage of staining techniques of low quality (Dobreva, 1980; Valkov, 1981; Eisenberg *et al.*, 1986; Grand *et al.*, 1986; Zahariev, 1995; Chaiwun *et al.*, 2002; Choi *et al.*, 2004). A false positive result can be obtained because of the presence of cellular atypism, caused by a reactive inflammatory process (Valkov, 1981; Tashev, 1982; Baker & Lumsden, 2000). False positive results are dangerous as they could be the cause for unnecessary surgical intervention, and risky irradiation or chemotherapy. A great percentage of the false positive diagnoses and errors in cytological conclusions could be avoided by improvements in the techniques of obtaining and staining of the cytological material. Despite the constantly expanding capabilities of cytological testing, the diagnostical value of the method has its limitations. One of the primary imperfections of punction cytodiagnostics is the anatomo-topographical limitation of the obtained material (Dobreva, 1980; Rosenthal, 1986; Menard & Papageorges, 1997).

The analysis of our results showed that, cytologically, it is not possible to determine the infiltrative or non-infiltrative nature of the neoplastic growth and the anatomo-topographical origins of the mammary neoplasias in bitches. Apart from that, the relation between the parenchyma and the stroma, as well as the relations between the epithelium and the underlying tissues, and, especially, the condition of the basal membrane cannot be determined. Cytologically, it can be established if the formation is benign or malignant, and whether it has epithelial, mesenchymal, or a mixed origin. Benign mammary neoplasias are cytologically identified by the same sizes and shapes of their

cells, which have similar nuclear-cytoplasmic ratios. The cells' nuclei are nearly the same, are stained in the same manner, and their nucleoli are usually not visualized. On the contrary, neoplastic cells of malignant mammary tumours have various shapes and sizes. The cells' nuclei are pleomorphic and atypical; the chromatin is unevenly distributed; often, there are nucleoli within the nuclei with varyous shapes and sizes (Alleman & Bain, 2000; Baker & Lumsden, 2000).

Cytologically, mammary gland tumours in female dogs can be divided into epithelial, mesenchymal, and mixed-type. This classification is based on the shape, size, and the disposition of neoplastic cells in the cytological samples (Tyler *et al.*, 1999; Alleman & Bain, 2000; Raskin, 2001).

Neoplastic cells in epithelial tumours are situated in separate groups, rarely together. Cytoplasmic boundaries are clear, and cell nuclei have round/oval shapes.

Mesenchymal tumours mainly consist of individually settled neoplastic cells, mixed with extracellular matrix. The shape of the cells is fusiform, stellate or oval, and their nuclei are oval/elliptic. The cellular content is mostly scarce.

Mixed mammary neoplasias consist of both epithelial and mesenchymal cellular elements, mixed with extracellular matter. The quantities of the mesenchymal and epithelial components vary in the different formations.

In conclusion, the cytological test could be used as an additional method in the diagnostics of mammary neoplasia in female dogs. The method is adequate for rapid diagnostics, yet the detailed classification of the canine mammary neoplasms at present requires the application of histological testing as well.

SPECIAL THANKS

We would like to express our gratitude towards the teams of the Department of Veterinary Surgery, the Department of Obstetrics, Gynaecology and Andrology at the Faculty of Veterinary Medicine, Trakia University, as well as all colleagues in the field who supported the completion of the current research.

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Paper received 25.04.2005; accepted for publication 04.07.2006

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