



CORRELATION BETWEEN FINE-NEEDLE ASPIRATION BIOPSY AND ROUTINE HISTOPATHOLOGY IN THE DIAGNOSIS OF SPONTANEOUS FELINE MAMMARY GLAND TUMOURS

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Summary

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The present study evaluated the correlation between fine-needle aspiration biopsy and routine histopathological examination in the diagnosis of spontaneous mammary gland tumours in cats. From the studied 120 neoplastic growths, cytological diagnosis was correct in 111 cases (92.5%), and incorrect in 9 (7.5%) patients. Out of all studied tumours, 3 false positive (2.5%) and 6 false negative (5%) results were found out. Among benign tumours, cytological diagnosis was correct in 17 cases (94.44%). Only one false negative diagnosis was demonstrated (5.56% of all benign tumours). As to malignant tumours, cytological diagnosis was correct in 94 (92.15%), and incorrect in 8 patients (7.85%). For diagnostics of feline mammary neoplasms, the sensitivity of cytology was 95.23%, the specificity – 75%, positive predictive value: 97.5% and negative predictive value: 60% compared to routine histopathology. The analysis with Cohen's kappa test showed agreement of 99.17% (Cohen's κ 0.96) between cytology and histopathology for benign and 94.17% (Cohen's κ 0.75) for malignant mammary neoplasms.

Key words: cats, correlation, fine-needle aspiration biopsy, histopathology, mammary tumours

INTRODUCTION

Mammary neoplasms in cats rank third after haematopoietic and skin tumours (Dorn *et al.*, 1968; Carpenter *et al.*, 1987; Misdorp *et al.*, 2002). The majority (85–90%) are malignant (Hayes *et al.*, 1981; Carpenter *et al.*, 1987). Mammary gland carcinomas affect more commonly adult animals, are outlined with high metastatic

potential and high mortality (Hayden & Nielsen, 1971; Hayden *et al.*, 1989; Misdorp, 2002; Hughes & Dobson, 2012). It is considered that the prognosis in adult cats is worse regardless of inconsistent research data. In cats <10 years of age, no correlation between age, recurrence and survival has been found (MacEwen *et al.*,

1984; Ito *et al.*, 1996). The major part of diseased animals are intact females, although spayed animals could be also affected (Hayes *et al.*, 1981; Weijer & Hart, 1983). A study reported that intact female cats were 7 times more likely to develop mammary gland tumours than spayed animals (Dorn *et al.*, 1968). Furthermore, female cats spayed during the first year of life, are less likely to develop mammary carcinomas (Overley *et al.*, 2005). The age of females affected with mammary gland tumours ranges from 9 months to 23 years, average 10–12 years (Weijer & Hart, 1983; Tomlinson *et al.*, 1984; Carpenter *et al.*, 1987; Ito *et al.*, 1996). Mammary neoplasms are observed also in tomcats with average age of onset 12.8 years (Skorupski *et al.*, 2005). The sex-related biological behaviour of neoplasms is similar (Skorupski *et al.*, 2005). Breed predisposition was established for Siamese and Oriental cat breeds (Gabor *et al.*, 2001; Louwerens *et al.*, 2005). In long-haired breeds as Persians, the prognosis of mammary neoplasms is poorer compared to that in other breeds (Weijer & Hart, 1983). In male cats, no association between breed and tumour behaviour was reported (Ito *et al.*, 1996; Skorupski *et al.*, 2005).

The cytological diagnosis of tumours is based on examination of single cells and cell complexes (Frale, 1983; Baker & Lumsden, 2000; Raskin, 2001). The main advantages of this method are the simplicity of preparation and rapid diagnostics. With rapid commercial staining kits, the diagnosis is often available within minutes, which makes express cytological test an important tool in contemporary medicine (Frale, 1983; Baker & Lumsden, 2000; Raskin, 2001). The rapid cytological identification allows an objective decision-making about the surgical inter-

vention, especially in cases with unclear pathology. In many cases, due to several reasons, the collection of material for histopathological examination from a site that is hard to reach is impossible. In such cases, cytology saves time particularly if the finding is small, mucous, haemorrhagic or necrotic (Cardozo, 1973; Dobрева, 1980; Berner *et al.*, 2003).

The role and importance of cytology as a fast diagnostic method in mammary gland tumours is under investigation from several decades. Melnik (1975) has identified correctly 624 out of 627 women with mammary neoplasms by cytology (99.52% accuracy). The only false negative result was attributed by the author to the small size of the neoplastic growth. According to other literature sources, the correlation of cytological test with ultimate histopathological diagnosis in women with mammary tumours was over 90% (de Guzman *et al.*, 2002; Yu *et al.*, 2002; Berner *et al.*, 2003; Choi *et al.*, 2004). Intra-operative cytological diagnostics could provide information whether the growth is malignant, for its typisation, determination of tumour margins and presence of local metastases (Cardozo, 1973; Dobрева, 1980).

The aim of the present study was to evaluate the correlation between fine-needle aspiration biopsy and routine histopathological examination in the diagnosis of spontaneous mammary gland tumours in cats in light of the fact that so far, results from similar studies on this type of neoplasms in Bulgaria were not reported.

MATERIALS AND METHODS

The studies were conducted on cytological and biopsy specimens from spontaneous mammary gland tumours from cats, re-

ferred for examination and treatment to the surgical clinic of the Faculty of Veterinary Medicine, Trakia University – Stara Zagora and private veterinary clinics in all regions of Bulgaria for the period 2000–2010. A total of 120 mammary gland tumours were examined (102 malignant and 18 benign). The examinations were done in the Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University – Stara Zagora. Neoplasms were identified in accordance with WHO International Histological Classification of Tumors in Domestic Animals (Misdorp *et al.*, 2001), completed by Histologic Classification of Feline Mammary Neoplasm (Meuten, 2017).

Specimens for cytology of all tumours were collected by means of fine-needle aspiration biopsy (FNAB) before surgery for removal of neoplastic growths. FNAB was done with 10 cm³ syringes and 20–22 G needles. The technique was as followed: the tumour growth was localised between the thumbs and the other fingers of the left hand, while the needle with the attached syringe was inserted into the mass. The syringe plunger was pulled back rapidly and vacuum was created. Maintaining the negative pressure, the needle was moved back and forth in different directions. Before removal of the needle, the plunger was returned to initial position to release the pressure. After the needle was withdrawn, the syringe was detached, air (2–3 cm³) was aspirated and cells inside the needle hub expelled onto a glass slide. Thus, the risk of cell damage and contamination with blood was minimised, but the amount of cellular material was not always sufficient. The centre of the neoplastic growth was avoided due to the risk of necrotic matter aspiration. When the cell material was not sufficient,

the manipulation was repeated in several areas of the tumour. This way, the risk for obtaining a poor-quality (haemorrhagic, necrotic) specimen was minimised. The flat side of another slide was placed under right angle vs the sample and rapidly smeared along the specimen slide for even distribution of cells from a thick into thin layer and good visualisation of the findings. Then, preparations were fixed with Merckofix-spray[®] (Merck[®], KGaA, Germany). Wet preparations were sprayed three times from a distance of about 20 cm (after evaporation of the alcohol, cells were protected by a thin polyethylene glycol film).

All cytological preparations were stained with Hemacolor[®] (Merck[®], KGaA, Germany). Mammary gland tumours were identified as benign or malignant according to the following cytological malignancy criteria (Tyler *et al.*, 1999):

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

- anisonucleosis: changes in nucleoli's size.

If three or more of the above listed malignancy criteria were met, the tumour growths were cytologically classified as malignant, and in all other cases – as benign. For determination of correlation of cytological diagnoses with ultimate histopathological diagnoses, the following indices were used:

- sensitivity = true positive diagnoses / (true positive + false negative diagnoses);
- specificity = true negative diagnoses / (true negative + false positive diagnoses);
- positive predictive value, PPV = true positive diagnoses / (true positive + false positive diagnoses);
- negative predictive value, NPV = true negative diagnoses / (true negative + false negative diagnoses).

For histopathological examination, specimens were fixed in 10% neutral formalin and processed by routine histopathological technique (Dyakov *et al.*, 1989). Cross sections of 4 µm were stained with haematoxylin/eosin. All cytological diagnoses were compared to the ultimate histopathological diagnoses. The comparison was only with respect to malignancy of neoplasms (benign vs malignant) and not regarding their detailed classification. The agreement between cytology and histopathology for benign and malignant tumours was tested by the Cohen's kappa test.

RESULTS

From the studied 120 neoplastic growths (Table 1), cytological diagnosis was correct in 111 cases (92.5%), and incorrect in

Table 1. Correlation between cytological and histopathological diagnoses with respect to malignancy (benign vs malignant) of examined neoplasms.

Tumours	Number	False positive	False negative	Correlation (%)
<i>I. Benign tumours</i>				
Adenoma	5	–	1	80.00
Fibroadenoma	13	–	–	100.00
<i>Total benign</i>	18	–	1	94.44
<i>II. Malignant tumours</i>				
Tubulopapillary carcinoma	60	1	1	96.66
Solid carcinoma	15	–	–	100.00
Cribriform carcinoma	7	–	1	85.71
Carcinoma in situ	1	–	–	100.00
Anaplasrtic carcinoma	1	–	–	100.00
Squamous cell carcinoma	10	–	2	80.00
Mucinous carcinoma	1	–	–	100.00
Spindle cell carcinoma	1	1	–	0
Inflammatory carcinoma	1	–	1	0
Fibrosarcoma	5	1	–	80.00
<i>Total malignant</i>	102	3	5	92.15
Total	120	3	6	92.50

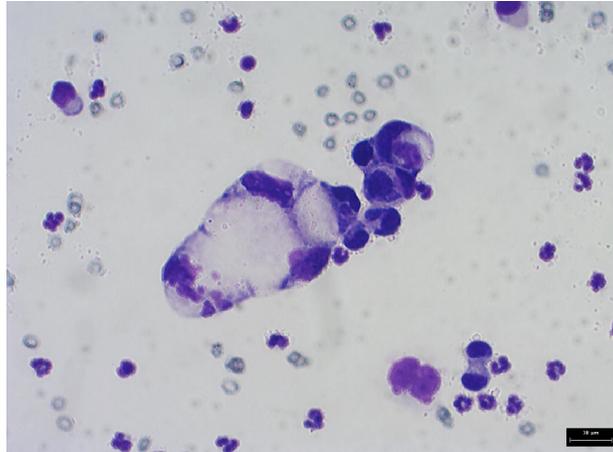


Fig. 1. Cytological finding in mucinous carcinoma. Irregular mitotic figures. Hemacolor[®] staining; bar=30 μ m.

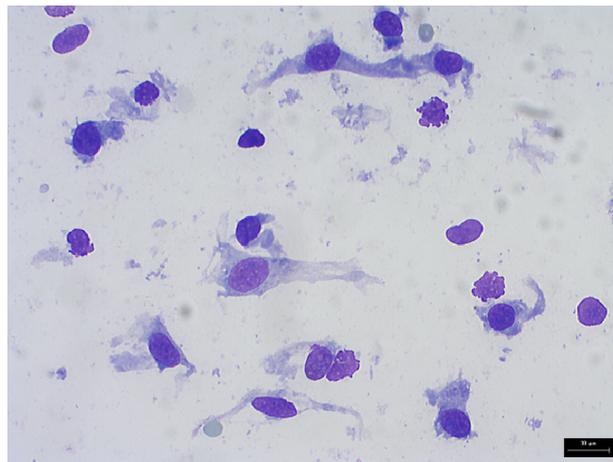


Fig. 2. Cytological finding in fibrosarcoma. Neoplastic cells are oval or fusiform, and arranged mostly individually. Hemacolor[®] staining; bar=30 μ m.

9 (7.5%) of studied neoplasms. Out of all studied tumours, 3 false positive (2.5%) and 6 false negative (5%) results were found out. Among benign tumours, cytological diagnosis was correct in 17 cases (94.44 %). Only one false negative diagnosis was demonstrated (5.56% of all benign tumours). As to malignant tumours, cytological diagnosis was correct in 94 (92.15%); in 8 patients (7.85%) it was

wrong (Fig. 1 and 2). Data about the agreement between cytological and histopathological diagnoses of malignant tumours showed that false positive and false negative diagnoses were respectively 2.94% and 4.9% of all malignancies.

For diagnostics of feline mammary neoplasms, the sensitivity of cytology was 95.23%, the specificity – 75%, positive predictive value: 97.5% and negative pre-

dictive value: 60% compared to routine histopathology. The analysis with Cohen's kappa test showed agreement of 94.17% (Cohen's κ 0.96) between cytology and histopathology for benign and 92.56% (Cohen's κ 0.75) for malignant mammary neoplasms.

DISCUSSION

In veterinary medicine, only few studies have investigated the reliability of cytological diagnosis of feline mammary gland tumours. One of them reported 100% correlation between cytological and histopathological diagnoses (Shafiee *et al.*, 2013). More studies were published regarding canine mammary tumours. Allen *et al.* (1986) have performed cyto-histological comparison of 91 mammary tumours from bitches. Cytopreparations were observed by two pathologists, which diagnosed correctly half of benign tumours and about one-fourth of malignant neoplasms. Cytological examination of 84 spontaneous mammary gland tumours from bitches have classified 21 tumours as malignant, 14 – as benign, and for another five, diagnosis was not provided (Hellmen & Lindgren, 1989). The results from this study demonstrated 79% accuracy of cytology vs ultimate histopathological diagnoses. In a study on 35 mammary gland tumours, Zuccari *et al.* (2001) reported 63% accuracy of cytology vs histopathology. Comparable results were published by Menard *et al.* (1986) for 6 mammary tumours, two of which were correctly diagnosed as malignant, three were suspected and for one, evaluation was impossible. In the view of researchers, the unsatisfactory results from cytological examination were due to poor quality of samples, especially the presence of necrotic matter and blood. An earlier study of ours

has investigated the correlation between cytology and histopathology of 70 spontaneous canine mammary tumours (Simeonov & Stoykov, 2006). The comparison was done only with respect to malignancy of neoplastic growths but not with respect to detailed classification of tumour lesions. Out of studied tumours, cytological diagnosis was correct in 57 (81.42%) and incorrect in 13 (18.57%) cases. From all studied tumours, 5 diagnoses were false positive (7.14%) and 8 (11.42%): false negative. Among 13 benign tumours, cytological diagnosis was correct in 11 (84.61%) cases whereas 2 diagnoses (15.38%) were false positive. For the 57 malignant tumours, 46 (80.7%) were correctly diagnosed. There were also 3 (5.26%) false positive and 8 (14.03%) false negative diagnoses.

In this study, the diagnostics of fibrosarcomas and squamous cell carcinomas were the most intricate. In the former type, cells were hardly exfoliated and thus, the cellular composition of findings was scarce. This was confirmed also by other researchers (Allen *et al.*, 1986; Menard *et al.*, 1986; Helmen & Lindgren, 1989; Zuccari *et al.*, 2001). Squamous cell carcinomas were accompanied by inflammation, which may result in inadequate interpretation of findings.

Most authors affirmed that the main reason for the false negative results was the improper sampling technique and the scarce amount of diagnostic material, whereas false positive results were associated with lack of experience, negligence or use of insufficiently good staining technique (Frable, 1983; Eisenberg *et al.*, 1986; Grant *et al.*, 1986; Chaiwun *et al.*, 2002; Choi *et al.*, 2004).

Despite the continuously increasing potential of cytology, the diagnostic value of the method is somewhat limited. One of

the main disadvantages of FNAB is the spatial restriction of specimen origin (Dobrev, 1980; Menard *et al.*, 1986). In our opinion, cytology is not capable to determine the infiltrative or non-infiltrative behaviour, the anatomo-topographic origin and detailed classification of mammary gland tumours in cats. What is more, the relationships parenchyma/stroma and epithelium/underlying tissues, as well as the condition of basement membrane cannot be determined. This is the cause for the lack of an uniform cytological classification of feline mammary tumours. Cytology can tell whether a specific growth is benign or malignant, and whether it is of epithelial or mesenchymal origin.

In conclusion, the cytological technique can be used as auxiliary method in the diagnosis of mammary gland tumours in cats. The method is suitable for express diagnostics, but histopathological examination is necessary for detailed classification of neoplasms.

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