Cytological Evaluation of canine mammary tumours with fine needle aspiration biopsy technique

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SUMMARY

Although cytopathology is widely used for early diagnosis of human tumours, it is not commonly performed in veterinary medicine. The aim of the present study was to compare cytological examination after pre-operative fine needle aspiration biopsies from canine mammary tumours (n = 31) with classical histopathology performed after surgery. Among the 26 available aspirates from various and heterogeneous mammary gland tumour masses, 20 exhibited atypical epithelial cells coupled to nucleus and/or chromatin anomalies, mitotic figures or spindle shape cells and were classified as malignant, 3 only showed modified epithelial cells and were considered as malignant suspected and 3 aspirates were considered as benign because epithelial cells appeared uniform. The most frequent histological types of tumours were malignant mixed tumours and adenocarcinomas, mainly tubular and papillary adenocarcinomas. The agreement score between the 2 techniques was 88.5%, the cytologically suspected malignant tumours being malignant by histology. These results suggest that pre-operative cytopathological examination of mammary masses may be helpful in the early malignancy diagnosis and in the therapeutic decision.

Keywords: Dog, Mammary tumour, cytopathology, histopathology, malignancy.

Introduction

Skin and mammary tumours are the most encountered tumours in dogs [17]. No geographical variation is identified for the existence and frequency of these tumours in worldwide reports. Due to acquired literature, mammary gland tumours are diagnosed in 25-30% of all the tumours in dogs and mixed tumours and carcinoma are diagnosed in 50-65% and 25-40% of cases of mammary tumours, respectively. The others are diagnosed as hyperplasia, adenoma and myoepithelioma [8, 12, 20]. Nevertheless, these percentages may vary according to the classification criteria [12, 17].

Mammary tumours are reported to occur rarely in young dogs (less than 2 years old): their incidence appears maximal in 6-7 years old dogs thereafter it decreases in 10-11 years old dogs [17]. Breed predisposition is unknown but it is suggested that dog mammary gland tumours are encountered more in breeds kept for hunting and outdoor such as Pointer, English setter, Retriever, Spaniel, Poodle, Boston terrier and Dachshund [12, 17]. These tumours are more often diagnosed in females than in males and beneath females more often in sterile individuals [16, 17].

Considering the lobe distribution of mammary gland tumours in dogs, the caudal lobe seems to be more often affected. Furthermore, 2 different types of tumours could be diagnosed in one or more lobes [8, 16, 18]. Mammary tumours may stem from myo-epithelial or luminal epithelial cells that form ducts and acini or from connective tissue that...
surrounds mammary ducts and acini. It is known that mammary tumours in dogs exhibit histological heterogeneity: most of the benign tumours are complex or mixed tumours [9, 10, 17].

Anamnesis and physical examination coupled with epidemiological findings have an important role in the diagnosis and prognosis. Radiographic screening, surgical biopsy or aspiration biopsy are beneficial tools for early diagnosis of tumours. Although the first attempt is to make a differential diagnosis with inflammation or hyperplasia these tools should be used to understand the biological behaviour of the mass that also contribute to the prognosis [2].

Cytological examination has important benefits in clarifying some aspects in early diagnosis of mammary lesions. This procedure is commonly used in palpable lesions such as mammary glands, thyroid, lymph nodes and salivary glands. It also prevents the need for a surgical attempt and complications that might occur during surgery [7]. Fine needle aspiration biopsy technique, speedy and cheaper than surgical biopsy [11, 14], has some disadvantages such as sampling of a low amount of tumour mass or inadequate tissues but these disadvantages might be minimized in experienced hands. It is also possible to prepare more aspirates in order to increase the number of representative cells as cell types and tumour morphology differ beneath lesions [2].

The aim of the present study is to compare the accuracy and the efficiency of a cytology technique, the fine needle aspiration biopsy technique (not commonly used for veterinary medicine in Turkey) with classical histopathological examination of mammary tumours.

Materials and Methods

Study material consisted of samples prepared from 31 lesions taken from various breed and aged 20 female dogs that were brought to the Gynaecology Clinics of Veterinary Medicine Faculty with the complaints of mass existence in different mammary lobes, between March 2007 and June 2008 (Table I). They were mainly terriers (11 dogs) or mixed (6 dogs), the 3 other animals were a Golden Retriever, an Irish setter and a Cocker. The average age was 11 ± 3 years (range values: from 6 years to 17 years); although the age was not known for 4 dogs, the majority of them (69%) were more than 10 years old.

The aspirated samples for cytology examination using fine needle aspiration biopsy technique were performed with the help of 10 ml injector and metal injector holder from dogs which were mildly sedated with diazepam (0.2 mg/kg, IV, Diazem, Deva) and ketamine HCl (5 mg/kg, IV, Ketamidor, Richter Pharma Ag) combination preoperatively. Slides prepared from the aspirates were stained with May-Grünwald Giemsa then examined under light microscope (OLYMPUS BX51). On the other hand, dogs were induced with propofol (4 mg/kg, IV, Propofol, Abbott) and anaesthetized with isoflurane (2-3%, Isoflurane, Adeka) and masses were surgically removed; after macroscopic evaluation, they were fixed in 10% neutral formaldehyde solution. Tissue samples taken are routinely processed and embedded in paraffin. All 5-6 µm thin sections were stained with haematoxylin and eosin. Some of the sections are also stained with Masson’s Trichrome for differential diagnosis.

<table>
<thead>
<tr>
<th>Case n°</th>
<th>Breed</th>
<th>Age</th>
<th>Tumour localisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Terrier</td>
<td>12</td>
<td>Right cranio-inguinal lobe</td>
</tr>
<tr>
<td>2</td>
<td>Terrier</td>
<td>14</td>
<td>Left lobes</td>
</tr>
<tr>
<td>3</td>
<td>Mixed</td>
<td>8</td>
<td>Left caudo-inguinal lobe</td>
</tr>
<tr>
<td>4</td>
<td>Terrier</td>
<td>14</td>
<td>Right cranio and caudo inguinal lobes / left caudo-inguinal lobe</td>
</tr>
<tr>
<td>5</td>
<td>Golden Retriever</td>
<td>10</td>
<td>Left cranio and caudo inguinal lobes</td>
</tr>
<tr>
<td>6</td>
<td>Irish Setter</td>
<td>10</td>
<td>Left cranio and caudo abdominal lobes</td>
</tr>
<tr>
<td>7</td>
<td>Terrier</td>
<td>12</td>
<td>Left cranio abdominal lobe</td>
</tr>
<tr>
<td>8</td>
<td>Terrier</td>
<td>-</td>
<td>Left caudo inguinal lobe</td>
</tr>
<tr>
<td>9</td>
<td>Terrier</td>
<td>10</td>
<td>Right caudo inguinal lobe</td>
</tr>
<tr>
<td>10</td>
<td>Terrier</td>
<td>6</td>
<td>Left cranio inguinal lobe</td>
</tr>
<tr>
<td>11</td>
<td>Mixed</td>
<td>-</td>
<td>Left caudo abdominal lobe</td>
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<tr>
<td>12</td>
<td>Mixed</td>
<td>-</td>
<td>Right caudo inguinal lobe</td>
</tr>
<tr>
<td>13</td>
<td>Mixed</td>
<td>9</td>
<td>Right thoracic lobe</td>
</tr>
<tr>
<td>14</td>
<td>Terrier</td>
<td>9</td>
<td>Left caudo inguinal lobe</td>
</tr>
<tr>
<td>15</td>
<td>Mixed</td>
<td>-</td>
<td>Right caudo inguinal lobe</td>
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<td>16</td>
<td>Terrier</td>
<td>10</td>
<td>Left cranio and caudo inguinal lobes</td>
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<td>17</td>
<td>Mixed</td>
<td>7</td>
<td>Right cranio inguinal lobe</td>
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<td>18</td>
<td>Terrier</td>
<td>17</td>
<td>Right caudo abdominal lobe</td>
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<tr>
<td>19</td>
<td>Terrier</td>
<td>14</td>
<td>Left cranio inguinal lobe</td>
</tr>
<tr>
<td>20</td>
<td>Cocker</td>
<td>14</td>
<td>Left caudo abdominal lobe</td>
</tr>
</tbody>
</table>

7 masses on left lobes (Right mammary gland lobes are removed before).

Table I : Signalment of female dogs included in the present study.
According to clinical anamnesis, cytopathological and histopathological findings, the collected materials were categorized into 5 classes: (1) benign, (2) benign-suspected, (3) malign-suspected, (4) malign and (5) inadequate.

Results

MACROSCOPIC ANALYSIS

The macroscopic characteristics of the different mammary masses were summarized in the Tables I and II. The preferential localisation of mammary tumours was the inguinal lobes (71% of cases), and especially the caudo-inguinal lobe (59% of the inguinal masses affected the caudo-inguinal lobe) whereas thoracic mass was found in only one case and abdominal masses in 25% of cases. Moreover, 60% of tumour masses were found in the left mammary lobes. Fifty-two % of mammary tumours exhibited a small size, with weight inferior to 50g but a relatively high proportion of these masses (38%) weighted more than 100g, even reaching 545g and 2500g (cases n° 13 and 16 respectively). The majority of the tumours showed a hard or an elastic consistency but some of them appeared fluctuant (cases n°1, 5 and 6). In the great majority of cases (76%), the aspect of the tumour on the cut surface was greyish or yellowish and lobed. Some cystic structures or necrotic areas were also often found (in 33% and 19% of cases, respectively).

CYTOPATHOLOGICAL FINDINGS

The pre-operative fine needle aspiration biopsy materials were not available in dogs n°10 and 15, and the slides prepared from the aspirates sampled from the dogs n° 6 (2 tumour masses) and 18 were inadequate for cytological analysis. The examination of the aspirates taken from 26 masses in the 16 remaining dogs (Table III) revealed malign in 20 masses, malign suspected in 3 and benign properties in the 3 last masses.

In all cases with malign characteristics (malign and malign suspected masses), clusters of cells with anisocytosis, anisokaryosis and hyperchromasia were observed (figure 1A). The mass was considered as malign suspected when no other criteria for malignancy was encountered (dogs n°1, 3 and 19). Nevertheless, necrotic cellular debris and erythrocytes were observed in tumour masses from dogs n°1 and 3. In the other malignant tumours, some nuclear anomalies were identified such as double nucleus (figure 1B) in 11 samples (55% of malignant tumours), giant nucleus in 10 samples (50%), mitotic figures (figure 1C) in 9 samples (45%) and abnormal chromatin structures (figure 1D) in 5 samples (25%). In 2 cases spindle shaped cells were associated with tumour cells (figure 1E). Necrotic cellular debris, some erythrocytes and neutrophil granulocytes were also sometimes found in the tumour mass (dogs n°5, 7 and 17). In the 3 benign tumours (one from the dog n°11 and 2 from the dog n°16), the mammary gland structure remained uniform and epithelial cells have formed bigger clusters (figure 1F) than those observed in malignant tumours. Besides, necrotic cellular debris and neutrophils were also found in the 2 masses from the dog n°16.

HISTOPATHOLOGICAL FINDINGS

The histopathological analysis (Table IV) have confirmed the cytological findings in the majority of cases: the 3 tumours considered as benign by cytology were one benign mixed tumour with embryonic connective tissue and cartilage tissue cells due to metaplasia (dog n°11) (figure 2A) and 2 cystic hyperplasia (2 tumours of the dog n°16), and the malignancy character was certified by histopathology in the 20 cytologically classified malign masses. The agreement score between cytological and histological classifications (number of identical results) was 88.5%. Furthermore, the histological examination has revealed the malignancy in the 3 suspected cases by cytology (dogs n°1, 3 and 19). Consequently, 90% of the 31 tumour masses investigated here presented malignancy. Among them, the most frequent type was malignant mixed tumours (15/28, i.e. 53.6%) (figure 2B) with embryonic connective tissue in 2 cases, and/or cartilage in 5 cases (figure 2C) or bone in 8 cases. The other type of malignant tumours encountered was adenocarcinoma.

![Figure 1](image-url)
(13/28, i.e. 46.4%): solid in 4 cases, papillary and tubulo-papillary with cholesterin clefts in 5 cases, tubular cystic with large cystic structures in 2 cases, myxomatous in one case (dog n°2) containing embryonic connective tissue and complex adenocarcinoma in one case (dog n°13). In this later, solid adenoid structures composed of atypical mammary gland epithelial cells were associated with fibroblasts and fibrocytes (figure 2D).

**CASE n°** | **TUMOUR DIMENSIONS | **WEIGHT (g)** | **CONSISTENCY** | **ASPECT OF THE CUT SURFACE**
---|---|---|---|---
1 | 216 g / 10 x 8.5 x 6 | Fluctuant | Greyish white with bloody cystic structures
2 | 43g / 7 masses from 0.8x0.5x0.5 to 2.5x1.5x1.2 | Elastic to hard | Generally greyish white with necrotic areas (1st and 7th masses)
3 | 15g / 1x0.7x0.5 | Hard | Greyish white and lobed
4 | Right: 80g / 2x1.3x0.7-1x1x0.5 | Hard | Greyish white and lobed
| Left: 20g / 6.2x4.5x3-3x3x2 | | |
5 | 124g / 3x3x3 | Elastic and fluctuant in some areas | Greyish white with a bloody cystic structure
6 | 220g / 2x1.5x1-14x12x4 | Elastic / Fluctuant | First mass: greyish white and lobed
| Second mass: cystic formations with yellowish brown mucous fluid
7 | 120g / 6x5.5x2.5 | Elastic | Greyish white and lobed with bloody cystic structures
8 | 101g / 7.5x4.5x4 | Hard | Greyish white, lobed with yellowish necrotic areas
9 | 87g / 3x2x2 | Hard | Greyish white and lobed
10 | 15g / 2x2x1 | Hard | Greyish white and lobed
11 | 28g / 4x3.5x2 | Elastic to hard | Greyish white, lobed with cystic structures
12 | 48g / 3x2x2 | Hard | Greyish white, lobed with reddish brown necrotic areas
13 | 545g / 14x14x6 | Hard | Yellowish, lobed with circular structures
14 | 12g / 2x1x0.5 | Hard | Yellowish brown with cystic structures filled with red coloured gelatinous fluid
15 | 272g / 10x9x8 | Elastic | Greyish white, lobed, with hemorrhagic areas
16 | 2500g / 25x20x13-11x7x5 | Elastic | Yellowish, lobed, with necrotic areas
17 | 20g / 6x4x2 | Hard | Greyish yellow, lobed, with hemorrhagic areas
18 | 13g / 2x2x2 | Hard | Greyish white and lobed
19 | 18g / 3x2x1 | Elastic | Yellowish and lobed
20 | 15g / 2x2x1.8 | Elastic | Yellowish white, lobed with cystic areas

**TABLE II :** Macroscopic characteristics of the mammary masses in the 20 females dogs included in the present study.
### Case / (number of masses) | Cytological criteria | Cytological class
--- | --- | ---
N°1 (1) | Anisocytosis + | MS
N°2 (7) | Double nucleus + (4) | M
N°3 (1) | Giant nucleus + (3) | M
N°4 (4) | Mitotic figure + (3) | M
N°5 (1) | Abnormal chromatin + (1) | M
N°6 (2) | Spindle shaped cells ND | ND
N°7 (1) | Uniform cells ND | ND
N°8 (1) | N°9 (1) | + (1) + (1) + (1) | M
N°10 (1) | + (1) + (1) | M
N°11 (1) | + | B
N°12 (1) | + | M
N°13 (1) | + | M
N°14 (1) | + | M
N°15 (1) | + | M
N°16 (2) | + (2) | B
N°17 (1) | + | M
N°18 (1) | + | M
N°19 (1) | + | MS
N°20 (1) | + | M

*M: Classified as malignant; MS: Classified as malignant suspected; B: Classified as benign.

**Table III**: Cytological analysis of pre-operative fine needle aspirates from the mammary masses of the 20 females dogs included in the present study. The number of masses identified in the same dog was indicated into parenthesis.

### Case / (number of masses) | Histopathological type | Histopathological classification
--- | --- | ---
N°1 (1) | Tubular cystic adenocarcinoma | Malignant
N°2 (7) | Solid / myxomatous / papillary adenocarcinomas (3) / Malignant mixed tumours (4) | Malignant
N°3 (1) | Malignant mixed tumour | Malignant
N°4 (4) | Tubulopapillary adenocarcinoma (1) / Malignant mixed tumours (3) | Malignant
N°5 (1) | Tubular cystic adenocarcinoma | Malignant
N°6 (2) | Papillary cystic adenocarcinoma (1) / Malignant mixed tumour (1) | Malignant
N°7 (1) | Solid adenocarcinoma | Malignant
N°8 (1) | Malignant mixed tumour | Malignant
N°9 (1) | Tubulopapillary adenocarcinoma | Malignant
N°10 (1) | Malignant mixed tumour | Malignant
N°11 (1) | Benign mixed tumour | Benign
N°12 (1) | Malignant mixed tumour | Malignant
N°13 (1) | Complex adenocarcinoma | Malignant
N°14 (1) | Malignant mixed tumour | Malignant
N°15 (1) | Solid adenocarcinoma | Malignant
N°16 (2) | Cystic hyperplasia (2) | Benign
N°17 (1) | Malignant mixed tumour | Malignant
N°18 (1) | Solid adenocarcinoma | Malignant
N°19 (1) | Papillary adenocarcinoma | Malignant
N°20 (1) | Malignant mixed tumour | Malignant

**Table IV**: Histopathological analysis of mammary tumour masses sampled during surgery in the 20 females dogs included in the present study. The number of masses identified in the same dog was indicated into parenthesis.
Discussion

The fine needle aspiration biopsy technique is a speedy, accurate and economic diagnostic tool used in the diagnosis of mammary tumours in humans. By contrast, this technique is still restricted in veterinary medicine, probably because of the lower frequency of diagnosis of mammary gland tumours in dogs compared to humans [3] and because of the large screening programs for breast cancers. In this study, comparison of fine needle aspiration cytology and histopathological examination is performed in order to determine the accuracy and efficiency of this technique in canine mammary tumours. With regard to the cases in which cytological sampling could not be performed, histopathological examination of these mentioned cases revealed progressive stage of the tumour, especially the ones showing solid structure or intense cartilage or bone tissue formations via metaplasia. The reason for the mentioned situation is thought to be late diagnosis of the tumour due to owner’s irrelevance.

In many studies, at least 10 criteria are usually used for stating malignant cellular characteristics such as variations of nucleus dimensions, giant nucleus formation, nucleus / cytoplasm distortion and rate, changes in chromatins structures (altered dimensions, irregular chromatins shapes in nucleus, clearing of the parachromatin areas), variation in nucleolus number, abnormal nucleolus shape and presence of macronucleolus, but it is also stated that commonly used criteria for malignancy are the nuclear dimensions and the nucleus / cytoplasm distortion [1, 5, 6, 13]. In the present study the main criteria retained to determine malignancy were the nucleus dimension and the variation of the nucleus / cytoplasm rate.

According to the cytological examinations in recent studies, canine mammary tumours were classified as malign, malign suspected and benign [4]. CASSALI et al. [3] found 67.5% of concordance between cytological and histological malignant classification for canine mammary tumours. In the current study, the agreement score between the 2 classifications was higher (88.5%), taking into account that the 3 cytologically suspected malignant cases were considered as negative results.

Most of the canine mammary tumours are benign or malign mixed tumours that are composed with epithelial and myoepithelial proliferations with generally cartilage, bone and squamous metaplasia [3]. ALLEN et al. [1] reported that the existence of spindle shaped cells within cytological aspirates should not be limited to mixed tumours as these cells might also exist in other mammary lesions such as myofibroblastoma. In the present study, spindle shaped cells were encountered in one case of malignant mixed tumour and in one case of complex carcinoma, agreeing in this way with the previous reports.

Tumour cells are located separately in fluids and secrets as they are less sticky than normal cells. These scattered cells are evaluated with the anaplastic properties of the originated tumour [14]. It is observed that preparation of multiple aspirates from various areas of the lesion increases the precision of the results despite the tumour heterogeneity: indeed, sampling should be performed from the peripheral places instead of the centre of the mass in which fluids and necrotic debris accumulate especially in big tumour formations [11]. Consistent with this, necrotic tissue remnants and inflammatory cells were identified in the slides prepared from the aspirates that were obtained from the areas close to centre of the large masses in the present study. Moreover, with animal owner’s awareness and cooperation, the tumour formations can be detected in an early stage by cytology and can be removed before progress. In progressed cases, as cytology leads to understand the biological behaviour of the masses, this analysis will help the clinicians to determine the type of treatment before surgery.

As already shown by other studies in veterinary medicine, fine needle aspiration cytology is one of the most interesting methods that provide conclusive and rapid diagnosis [15]. Even in cases in which the diagnosis is not definitive, the fine needle aspiration features may guide the clinical and surgical management. However, good sampling and adequate training in cytopathology are emphasized to be essential to get clinically relevant results [3]. In a recent study [19], a series of 190 breast masses were identified during the study period. The fine needle aspiration cytological diagnosis was unsatisfactory due to inadequate specimens in eight cases (4.2%). The diagnoses in the remaining 182 cases were: benign lesions in 98 (53.9%); suspicion for malignancy in 31 (17.0%); and malignant in 53 (29.1%). From the subsequent histopathological diagnoses, 6/98 cases of benign cytology turned out to be malignant lesions (false negatives); 22/31 cases of suspicious cytology were truly malignant while the other nine were benign; and only 1/53 with malignant cytology was benign (false positive). According to the results of this study clinicians are advised to correlate fine needle aspiration cytological results with physical examination and imaging findings to prevent false negative and false positive events and to obtain optimal management for their patients. Consequently, for establishing medical treatment or prognosis, we certainly suggest validation of cytological diagnosis with histopathological examinations.

As a conclusion, the cytological fine needle aspiration biopsy technique, which is unfortunately still not a common diagnostic tool in veterinary medicine, can easily be used routinely in early diagnosis of mammary gland and other palpable tumours. This study is hoped to open the way up for further cytopathology studies.

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