Cytologic Features of Testicular Tumours in Dog

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Summary

In this paper, we report on our experience of cytology of fine needle biopsies performed on 92 dogs with testicular tumours during the period from 1998 to 2002. Cytological diagnosis was consistent with seminoma in 20 cases, sertolioma in 16 cases, Leydig cell tumours in 50 cases and mastocytoma in one case. Five cases could not be diagnosed by cytology. Cytological observations were confirmed after surgery by histopathological examination in 87 cases. Cytology provided a sensitivity of 95% for seminoma, 88% for sertolioma and 96% for Leydig cell tumours. The specificity was 100% for all three tumour types. In our experience cytology of fine needle aspirations of testicular tumours is a very reliable technique.

Introduction

Testicular neoplasms are frequent in aged dogs (Hayes and Pendergrass, 1976) with seminoma, Sertoli cell tumours or sertolioma, and Leydig cell tumours being the most prevalent types of testicular neoplasia (Cohen et al., 1974; Hayes and Pendergrass, 1976), although cases of mixed germ cell tumours (Patnaik and Mostofi, 1993) and mesenchymal tumours (Herron, 1982) have been described. Older dogs are commonly affected by testicular enlargement (Scully and Coffin, 1952; Cotchin, 1960; Reif et al., 1979), especially in cryptorchid testes, where the incidence of neoplasia is higher compared to solitary lesions were submitted to cytological examination in 50 cases and mastocytoma in one case. Five cases could not be diagnosed by cytology. Cytological observations were confirmed after surgery by histopathological examination in 87 cases. Cytology provided a sensitivity of 95% for seminoma, 88% for sertolioma and 96% for Leydig cell tumours. The specificity was 100% for all three tumour types. In our experience cytology of fine needle aspirations of testicular tumours is a very reliable technique.

Materials and Methods

A total of 122 canines with testicular neoplasms underwent ultrasound examination of the gonads over a period of 4 years (1998–2002); the average age at the time of presentation ranged from 8 to 15 years; 89 of these dogs were mongrels, while the remaining cases included 12 German Shepherds, six Siberian Huskies, five Labrador Retrievers, four Yorkshire Terriers, three English Setters, one Doberman, one Samoyede, and one Chow-Chow; all dogs were presented for indolent testicular swelling; four cases had undergone previous antibiotic therapy without resolution of clinical symptoms. In all patients ultrasound-guided FNCS biopsy was performed (Mair et al., 1989; Yue and Zheng, 1989) on masses localized in the abdomen (nine cases), in the subcutis of the abdomen and parapenien region (18 cases) or in the descended testicle (95 cases). Ultrasound examination detected the presence of two or more distinct lesions in the same testicle in 30 patients.

For this study, testicles affected by two lesions were excluded and only samples from testicles with ultrasound-confirmed solitary lesions were submitted to cytological examination in order to avoid confusion between the cytological features and the presence of two or more neoplasms. The FNCS was consistent with seminoma in 20 cases, sertolioma in 16 cases, Leydig cell tumours in 50 cases and mastocytoma in one case. Five cases could not be diagnosed by cytology. Cytological observations were confirmed after surgery by histopathological examination in 87 cases. Cytology provided a sensitivity of 95% for seminoma, 88% for sertolioma and 96% for Leydig cell tumours. The specificity was 100% for all three tumour types. In our experience cytology of fine needle aspirations of testicular tumours is a very reliable technique.

Cytology of fine needle aspirations of testicular tumours is a very reliable technique. In this paper, we report on our experience of cytology of fine needle biopsies performed on 92 dogs with testicular tumours during the period from 1998 to 2002. Cytological diagnosis was consistent with seminoma in 20 cases, sertolioma in 16 cases, Leydig cell tumours in 50 cases and mastocytoma in one case. Five cases could not be diagnosed by cytology. Cytological observations were confirmed after surgery by histopathological examination in 87 cases. Cytology provided a sensitivity of 95% for seminoma, 88% for sertolioma and 96% for Leydig cell tumours. The specificity was 100% for all three tumour types. In our experience cytology of fine needle aspirations of testicular tumours is a very reliable technique.
order to avoid cellular disruption, and cells were simply allowed to enter the needle. The procedure was repeated several times to ensure that an adequate amount of material was collected. A total of 92 samples were then selected and submitted to cytological examination. Air-dried smears were stained by the May–Grünewald–Giemsa method. The smears were cytologically scored as poorly, adequately or highly cellular. An attempt was made to classify all neoplasms identified in the aspirate as seminoma, sertolioma or Leydig cell tumours using criteria previously described (De Nicola et al., 1980; Zinkl, 1999; Baker and Lumsden, 2000; Henson, 2001). After cytological diagnosis, all the dogs were submitted to surgical orchietomy; histopathological reports were available for all 92 patients. Macroscopically, all testicles were affected by a single parenchymal mass, which was immediately fixed in 10% neutral buffered formalin; 4-μm sections were cut from paraffin blocks prepared by routine procedures and stained with haematoxylin–eosin method. These tumours were histologically classified according to the WHO criteria (Kennedy et al., 1998). As negative control, fine-needle aspiration of 38 normal canine testicles from routine castrations was performed; cytological examination was executed in a blinded fashion. Immediately after surgical excision, each testicle was then submitted to a 25-G needle aspiration. The smears were collected as described for pathological testicles, and were air dried and stained with the May–Grünewald–Giemsa method. Normal testicles were fixed in 10% neutral-buffered formalin and submitted to histological examination. The cytological features observed for each tumour were reviewed and correlated with the definitive histological diagnosis.

Statistical analysis

The results of cytological and histological examination provided the numbers of true-negative (TN), true-positive (TP), false-positive (FP) and false-negative (FN) cases. The data were correlated in order to obtain the value of specificity, using the formula \( \text{specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \) and sensitivity using the formula \( \text{sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \). Multiple correspondence analysis (MCA) was used to explore the relationship between cytological aspects, categorized in two levels (present = 1; no present = 0) and histological type. The MCA is a weighted principal component analysis of a multivariate table that gives a low-dimensional graphical representation of all the levels of each categorical variable (Greenacre, 1984). Each level is represented by a point in Euclidean space in such a way that the distance between any two points may be interpreted in terms of strength of association between the corresponding categories. In addition, the points corresponding at different levels of the same variables are plotted at a distance from the origin that is inversely related to their weight (‘weight’ is expressed by the number of cases in the corresponding level). This method permits the representation in the same Euclidean space, not only of the variables used for its definition (active variables), but also of other variables provided on the same subjects (supplementary variables). In this study, we considered the cytological characteristics as active variables for analysis, and the histological type as a supplementary variable. The MCA was performed using STATA software, version 7.0 (Foundation for Statistical Computing, Vienna, Austria); for graphical representation of Euclidean space the r-software was employed. A single case of mastocytoma was excluded from this analysis.

Results

The results are summarized in Table 1. Cytological examination established a diagnosis of testicular tumour in 87 of the 92 patients. In two cases the aspiration was haematic and not diagnostic; in one case the aspiration sampled only normal testicular cells, represented by immature spermatozoa, germinal cells and Sertoli cells; in one case the aspiration was acellular and unsatisfactory; in one case only blood and low numbers of inflammatory cells were collected. The remaining smears were diagnosed as seminoma \((n = 20)\), sertolioma \((n = 16)\), interstitial tumours \((n = 50)\) and mastocytoma \((n = 1)\). Of 92 surgical removed tumours, 87 showed good correlation between the cytological findings and histological examination, as shown in Table 2. Two bloody smears, one acellular sample, and one bloody and inflammatory specimen were misdiagnosed as interstitial cell tumours, a seminoma and a sertolioma respectively. No cases of teratoma, mixed germ cells tumour or gonadoblastoma were observed by the veterinary pathologist.

With the exclusion of a single case of mast cell tumour, the frequency of the three tumour types in our 86 cases was 23.2% seminoma, 18.6% sertolioma and 58.1% interstitial cell tumours. These observations correlate well with the published data (Reifinger, 1988), particularly regarding the high prevalence of Leydig cell tumours, which represented about 50% of the present series (Dow, 1962; Ogilvie and Moore, 1995).

Microscopic features of smears from 20 seminoma presented neoplastic cells with variable amounts of bluish cytoplasm, and the presence of macrovacuoles was observed in only three cases (15%). Nuclei were large and round, sometimes with irregular outlines. Chromatin was granular and irregularly clumped in six cases (30%). One or more large nucleoli were present in 18 cases (90%). Numerous and atypical mitoses were observed in 18 cases (90%). Besides the neoplastic population, a diffuse proliferation of a number from moderate to high of mature lymphocytes was evident in all 20 seminoma (100%); these cells were recognizable by the small amount of cytoplasm, the round nucleus with clumped chromatin and by the small size of the cells compared with the voluminous neoplastic cells having a large nucleus, more irregular chromatin and a prominent acidophilic central nucleolus. Lacy, granular eosinophilic material with the appearance of a tigroid background was observed in six tumours (30%); a bloody background was present in 14 cases (70%). In all 20 seminoma an inflammatory infiltrate, represented by a variable number of plasma cells as well as neutrophilic and eosinophilic granulocytes, was observed.

The cytological features of the 16 cases of sertolioma showed large cells with abundant, macrovacuolated and

<table>
<thead>
<tr>
<th>Cytology report</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory specimen</td>
<td>1</td>
</tr>
<tr>
<td>Normal testicular cells</td>
<td>1</td>
</tr>
<tr>
<td>Bloody specimen</td>
<td>2</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>1</td>
</tr>
<tr>
<td>Seminoma</td>
<td>20</td>
</tr>
<tr>
<td>Sertolioma</td>
<td>16</td>
</tr>
<tr>
<td>Leydig cell tumour</td>
<td>50</td>
</tr>
<tr>
<td>Mastocytoma</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
</tr>
</tbody>
</table>
Table 2. Cytological typing of 87 testicular tumours and five non-diagnostic specimens, with histological correlation in 92 cases

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Cytological diagnosis of seminoma</th>
<th>Cytological diagnosis of sertolioma</th>
<th>Cytological diagnosis of Leydig cell tumour</th>
<th>Bloody smear</th>
<th>Normal testicular cells</th>
<th>Bloody smear and inflammatory cells</th>
<th>Mastocitoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminoma</td>
<td>20</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sertolioma</td>
<td>16</td>
<td></td>
<td>50</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial cells tumour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastocitoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

The cytological features of the 50 Leydig cells tumours were poorly defined (two cases, 12.5%), round (eight cases, 50%) to columnar profiles (six cases, 37.5%). These cells were present in groups, nests and cords; an amorphous granular background was evident in eight cases (50%) associated with a small number of erythrocytes in 10 cases; cytoplasmic fusion and palisading formation was evident in 11 of 16 cases (68.8%), expressed from the one beside the other arrangement of the neoplastic columnar cells. In all 16 cases the cells showed a macrovacuolated cytoplasm (100%), while a small number of microvacuoles were observed in four cases (25%). In one case (6.3%) only rare bluish cytoplasmic granules were evident. The nuclei showed only occasional nucleoli (four cases, 25%) and fine granular chromatin, which (25%) were irregularly clumped in four cases. A low number of atypical mitoses were observed in six cases (37.5%). Inflammatory cells were a consistent feature in 10 cases (62.5%); the presence of rare lymphocytes was observed in only four cases (25%).

The cytological features of the 38 normal testicles included a high number of immature spermatozoa, characterized by eosinophilic nuclei and short tails; these cells were associated with a large number of round-shaped germinal cells, with coarse nuclear chromatin and single prominent nucleoli, in high mitotic activity. A few groups of columnar cells, with indefinite cytoplasm and large round basal nucleus with a single nucleolus, recognizable as Sertoli cells, were evident. Scattered stellate or caudate Leydig cells, with a microvacuolated cytoplasm and round hyperchromatic nuclei, were sometimes detected on the smears.

The cytological features observed in the 86 testicular tumours are summarized in Table 3. Cytological examination provided a sensitivity of 95% for seminoma, 88% for sertolioma and 96% for Leydig cell tumours; the specificity was 100% for all three tumour types, as no FP specimens were observed.

A graphical representation in Euclidean space of the MCA is shown in Fig. 1. A variability of 79.1% is provided by the first dimension, expressed in the abscissa (d1), which separates interstitial from gonadostromal tumours; the second dimension, represented in the ordinate (d2) explains a 20.4% variability and permits differentiation between seminoma and sertolioma; simplification in two dimensions of the distribution of the cytological features leads to a 99% observed variability in the relationship among the different variables.

Table 3. Number of cases for the observed cytological features

<table>
<thead>
<tr>
<th>Cytological features</th>
<th>Abbreviations used in graphic</th>
<th>Seminoma (n = 20)</th>
<th>Sertolioma (n = 16)</th>
<th>Leydig cell tumour (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round shape</td>
<td>Rou</td>
<td>20</td>
<td>8</td>
<td>26</td>
</tr>
<tr>
<td>Spindle shape</td>
<td>Sp</td>
<td>0</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>Columnar shape</td>
<td>Col</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Palisading arrangement</td>
<td>Pal</td>
<td>0</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Perivascular arrangement</td>
<td>Periv</td>
<td>0</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>Fusion of cytoplasmic margin</td>
<td>Fucy</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Macrovacuolated cytoplasm</td>
<td>Macv</td>
<td>3</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>Microvacuolated cytoplasm</td>
<td>Micv</td>
<td>0</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>Blushing cytoplasm granules</td>
<td>Blug</td>
<td>0</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>Infl</td>
<td>9</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Tigroid background</td>
<td>Tigba</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytic infiltrate</td>
<td>Lym</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Nuclear pseudoinclusions</td>
<td>Psinc</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Atypical mytoses</td>
<td>Atym</td>
<td>17</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Amorphous background</td>
<td>Ambak</td>
<td>6</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Bloody background</td>
<td>Bloback</td>
<td>14</td>
<td>10</td>
<td>48</td>
</tr>
<tr>
<td>Macronucleoli</td>
<td>Macnu</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Irregular clumped chromatin</td>
<td>Irrcro</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
The distribution in Euclidean space of the features determine an area for any single tumour, in which a single feature is typical for any tumour type, the further it is from the origin of the axis.

Discussion

To the best of our knowledge, there are no published reports on FNCS of testicular tumours in dog, with the exception of isolated descriptions that have appeared in a few publications or in textbooks of veterinary cytology (De Nicola et al., 1980; Zinkl, 1999; Baker and Lumsden, 2000; Henson, 2001). Although there are several other types of testicular tumours, the most prevalent are seminoma, sertolioma and Leydig cell tumours (Cohen et al., 1974; Hayes and Pendergrass, 1976). No cases of teratoma, mixed germ cell tumours or gonadoblastoma were detected either by cytological or histological examination, although some descriptions in dogs have been previously reported (Reifinger, 1988).

As shown in Figure 1, the distribution of the observed features strongly relates seminoma with the presence of tigroid background (tigba), lymphocytic infiltrate (lym), macronucleoli (macnu) and atypical mytoses (atym). Sertolioma is strongly related to the fusion of cytoplasmic margins (fucy), the presence of a palisading arrangement (pal), columnar cell shaped (col) and with cytoplasmic macrovacuolation (macv). Interstitial cell tumours are strongly correlated with the presence of a perivascular arrangement (periv), cytoplasmic microvacuolation (micv), bloody background (bloback), spindle shape of the cells (sp) and localization of bluish cytoplasmic granules (blug).

The results of MCA, provided by the Standards for Reporting Diagnostic Accuracy (STARD) group (Bossuyt et al., 2003), suggest that cytological diagnosis can be very accurate in the correct tumour classification, because different types of tumours have very different cytological characteristics. High sensitivity (95% for seminoma, 88% for sertolioma and 96% for Leydig cell tumours) and specificity (100% for all the three types of neoplasms) allow us to conclude that cytological examination a very useful tool for clinical management of testicular neoplasms.

Discrete round-shaped neoplastic cells are dominant in seminoma, as previously described (Caraway et al., 1995). Only a low percentage of seminoma showed cytoplasmic vacuolation, generally present as round large vacuoles: many reports have documented this feature in humans, where both vacuolations and a tigroid background are evident, by periodic acid Shiff (PAS) staining, as glycogen (Fleury-Feith and Bellot-Besnard, 1989; Orell et al., 1992; DeMay, 1996; Bibbo, 1997). Our data confirm the previously published results (Caraway et al., 1995) and underline the infrequent presentation of a tigroid background (Fig. 2), expressed by the fragile cytoplasm of neoplastic cells spread over the slide that was detected in only six cases of seminoma. This explanation is probably related to the less traumatic collection procedure, employing a small needle and avoiding aspiration, which correlates with a small number of broken cells and consequently, lesser amounts of tigroid substances. Nevertheless, this feature appears to be indicative of seminoma, as it is totally absent in other neoplasms. Lymphocytic infiltrates, as previously described (Caraway et al., 1995), were detected in all cases of seminoma (Fig. 3), and seems to be a diagnostic hallmark of this tumour type. Although some reports mention the possibility of detecting it in sertolioma (Henley et al., 2002), this feature was observed in only a low percentage of our Sertoli cell (four cases, 25%) and Leydig cell tumours (two cases, 4%), where occasional and solitary mature lymphocytes

![Graphical representation of the multiple correspondence analysis showing the relationship between cytological aspects and histological type. LYD, Leydig cell tumours; SM, seminoma; ST, sertolioma.](image-url)
were scattered on the background of the smears. Nuclear features of malignancy, present as irregular clumped chromatin, the presence of macronucleoli (Fig. 4), and atypical mitotic figures (Fig. 5) were frequently observed in our seminomas but, despite the presence of these markers of malignancy, no metastatic behaviour was detected in the 20 cases of seminoma examined; this is probably related to the absence of infiltration of parenchyma, as observed in the histological sections and in agreement with the low rate of metastasis reported for this tumour type (Ogilvie and Moore, 1995; Cooley and Waters, 2001). No important features of nuclear malignancy were observed in any case of Sertolioma or Leydig cell tumours, where nuclei showed only irregularly clumped chromatin in a small percentage of both types of tumours.

Sertoliomas exhibit alternatively round or columnar shapes, but in 15 cases (93.8%) cytoplasmic fusion with a dominant microscopic pattern of sheet-like or trabecular disposition of the neoplastic cells was evident (Henley et al., 2002) (Fig. 6). These features have not been described in previous reports,
although ultrastructural examination of neoplastic cells revealed prominent intercellular gap junctions, providing a diagnostic hallmark of sertolioma (Ladds, 1993). Moreover, the sertolioma showed a palisading arrangement in 11 cases, as a direct expression of the disposition in histological sections, where cells tend to palisade along septa of fibrous stroma (Ladds, 1993; MacLachlan and Kennedy, 2002) (Fig. 7). Other reports have described this feature in Sertoli cell tumours, associated with arrangements in nests and tubules (Nijhawan et al., 1992). This architectural feature represents an important differential criterion with sertolioma, where cells are adhesive and organized in cohesive sheets, trabeculae and palisades; in seminoma single round cells are prominent. Only one case of sertolioma showed a small amount of cytoplasmic basophilic granulation, previously described and interpreted as lipochrome pigment (MacLachlan and Kennedy, 2002).

Cells from Leydig cell tumours frequently exhibited spindle-shaped cells and had a tendency to show a perivascular pattern, features that were frequently observed (44 cases, 88%) as previously reported (De Nicola et al., 1980) (Fig. 8). No perivascular arrangement was observed in other tumour types, making this feature a distinguishing hallmark in diagnosis of Leydig cell tumours. This can be explained by the close relationship between Leydig cells and vascular structures of the interstitial places, where the tumour originates. Cytoplasmic vacuolation is a prominent feature in Leydig cell tumours (Fig. 9), where cells show microvacuolization in all cases. This feature is also present on the background of the smears as consequence of cellular rupture. In Leydig cell tumours, vacuolation is interpreted by some authors as intracytoplasmatic accumulation of lipid production (De Nicola et al., 1980; MacLachlan and Kennedy, 2002). This aspect allows useful differentiation with Sertoli cell tumours, which show cytoplasms that are often filled with a few, large irregular vacuoles (Henson, 2001) (Fig. 10). These are frequently recognized by PAS staining as intracytoplasmic glycogen (Henley et al., 2002) and represent an important distinctive feature in differential diagnosis with seminoma, where vacuoles are rare or absent. A variable amount of basophilic granules were present in a high number of cases of Leydig cell tumours.
tumours (26 cases, 52%) (Fig. 11), previously described as lipofuscin or lipochrome pigment (De Nicola et al., 1980; Zinkl, 1999), and also well recognized in the histological sections (Ladds, 1993); this feature has not been described in other neoplasms, with the exception of a single case of sertolioma, where we interpreted this observation as an occasional localization of cytoplasmic lipochrome pigment (MacLachlan and Kennedy, 2002).

Nuclear pseudoinclusions were detected in 22 cases of Leydig cell tumours (44%) (Fig. 12) and absent in other types of neoplasms, appearing as small vacuoles embedded in chromatin material, which causes nuclear enlargement. These inclusions have been well investigated by electron microscopy and represent intranuclear cytoplasmic invaginations, which are strongly PAS positive and composed of smooth and rough endoplasmic reticulum, vesicles and lipid vacuoles, myelin figures and disrupted membrane profiles; this feature has not been observed in other testicular tumours (Ladds, 1993) and represents a diagnostic hallmark of Leydig cell tumours.

Conclusions

Our observations agree well with previously published data, while the presence of undescribed features improve the value of cytological investigation; on the basis of statistical analysis by MCA, the typical features of seminoma are round-shaped cells, round nuclei with macronucleoli, and the presence of lymphocytic infiltrate. Palisading arrangements, macrovacuolations and the fusion of cytoplasm, with palisade formations, are diagnostic of sertolioma and a perivascular arrangement, bloody background and microvacuolation are highly diagnostic of interstitial cell tumours. The application of these criteria permits sensitive and specific discrimination for the three most frequent neoplastic types of testicular tumours and provide a useful tool in their clinical management.

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