Use of Oil Red O stain in the cytologic diagnosis of canine liposarcoma

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Background: Oil Red O, a stain commonly used to demonstrate lipid in frozen tissue, also may be used to stain air-dried cytologic specimens. Objective: The purpose of this study was to prospectively evaluate the value of Oil Red O in identifying lipid to aid in the differentiation of liposarcomas from other types of sarcoma. Methods: Twelve tumor specimens from dogs were evaluated. The tumors were included in the study if initial cytologic evaluation indicated a sarcoma, and if histologic confirmation was available. Oil Red O was applied to all cytologic specimens. Results: Tumor specimens were diagnosed histologically as liposarcoma (3 well-differentiated, 1 pleomorphic), hemangiopericytoma (n = 3), fibrosarcoma (n = 3), malignant fibrous histiocytoma (n = 1), and undifferentiated sarcoma (n = 1). Cytologic specimens from all liposarcomas showed strong positive staining of cytoplasmic vacuoles for lipid. Specimens from other sarcomas stained negative for Oil Red O, with the exception of weak, irregular positive staining in 1 hemangiopericytoma. Conclusions: Our results suggest that Oil Red O staining may be an easy, inexpensive, and useful diagnostic tool for the differentiation of liposarcoma from other mesenchymal neoplasms. (Vet Clin Pathol. 2006;35:37–41)

Key Words: Dog, liposarcoma, mesenchymal tumors, special stains, Oil Red O

Liposarcoma is an uncommon tumor in animals and develops predominantly in adult dogs. The neoplasm arises spontaneously and is not derived from either of its benign counterparts, namely, lipoma or infiltrative lipoma. Diagnosis is based on the results of light microscopy of histologic sections and on ultrastructural features. Cytologic features of liposarcoma have been reported as ovoid to spindle cells with small to large lipid globules in the cytoplasm. In some cases, variable morphologic characteristics may be found in different areas of the same tumor, similar to what is frequently found in other sarcomas, this is especially true for less differentiated neoplasms, and hampers definitive cytologic diagnosis.

To the author’s knowledge, only Sudan III, Sudan IV, Sudan Black, and Oil Red O stains are available to demonstrate lipid in a neoplasm originating from adipose tissue. These stains can be used only on fresh or frozen tissue since lipids are removed by standard tissue-processing procedures. This may limit their use, as most surgically removed tissue specimens routinely are placed into formalin and embedded in paraffin for histologic examination. Cytologic smears, which typically are air-dried rather than fixed in alcoholic preservatives, represent a possible source of cells for use in special staining for the detection of lipids. Evaluation of cytologic specimens using Oil Red O could allow for the morphologic differentiation between liposarcoma and other mesenchymal tumors, and would permit the prior establishment of staining protocols or fixation procedures for surgically removed tissue specimens. The purpose of this study was to use Oil Red O as an adjunct stain in the cytologic diagnosis of sarcomas, and to compare the results with the histopathologic diagnosis.

Materials and Methods

Subcutaneous masses from 12 dogs during the period September 2001–April 2004 were evaluated. The tumors were prospectively selected if the cytologic results were indicative of sarcoma. Only masses for which a definitive histopathologic diagnosis subsequently was obtained were included in the study. The masses were aspirated by use of a fine-gauge needle, and at least 4 cytologic smears were prepared from each lesion. Air-dried smears were stained with May–Grünwald Giemsa (Carlo Erba Reagenti, Milan, Italy) and with Oil Red O (Diapath Microstain, Martinengo, Italy). Briefly, without fixation in alcohol, the smears were covered with a few drops of Oil Red O stain (that had been diluted 1:0.6 with distilled water) for 5 minutes, according to the method of Johnson. The slides were then rinsed in running tap water and counterstained by immersion for 5 minutes in Mayer’s hematoxylin (Diapath Microstain, Martinengo, Italy). After rinsing again in running tap water and air drying, the slides were covered overnight with a drop of aqueous mounting fluid (Crystal Mount, Biomeda, Foster City, CA, USA), then covered with balsam (Eukitt, Electron Microscopy Sciences, Hatfield, PA, USA) and a coverslip. After cytologic examination and diagnosis, all neoplasms were surgically removed and tissue specimens were submitted for histologic examination.
specimens were fixed, processed, and stained routinely with H&E. The cytologic diagnosis and results of Oil Red O staining were compared with the final histopathologic diagnosis.

Results

Four cases of liposarcoma were diagnosed based on both cytologic and histologic specimens (Table 1). The cytologic samples had good to excellent cellularity. The cells were arranged in a perivascular pattern, with the neoplastic cells organized around a vascular axis. Generally, the neoplastic cells had an indistinct spindle shape; cells in 1 case had an ovoid shape. The cells had abundant, slightly eosinophilic cytoplasm that often was filled with round, sharply demarcated vacuoles (Figure 1). High numbers of large vacuoles were seen in cells from 3 tumors, whereas cells in 1 tumor had smaller, fewer vacuoles. Abundant lipid-like material also was evident in the background of the smears. In samples from 3 of the 4 tumors, large ovoid cells were observed in which the cytoplasmic vacuolation deformed the profile of the nucleus, consistent with lipoblasts. In samples from 3 tumors, nuclei were round to oval, with finely clumped chromatin. In samples from the remaining case, anisokaryosis was moderate and nuclei had clumped chromatin with macronucleoli. Binucleated cells were found in specimens from all 4 cases.

The histologic features in specimens from 3 of the 4 cases of liposarcoma were consistent with the proliferation of ovoid or spindle cells with abundant, macrovacuolated cytoplasm and round to oval nuclei with finely stippled chromatin. Cells were arranged in large lobules supported by a stromal component (Figure 2). Vascular capillary structures were present in all tissue sections. One tumor had less differentiated lobular structures, in which large round cells with variable amounts of microvacuolated cytoplasm, anisokaryosis, and large, round nuclei containing clumped chromatin were evident.

In cytologic samples from all 4 liposarcomas, Oil Red O staining resulted in red-orange coloration of both the intracytoplasmic and extracellular vacuoles (Figure 3), demonstrating their lipid content. The intensity of staining was highly variable, ranging from complete to partial or peripheral staining of the large globules; cells in 1 sample had fewer smaller vacuoles (Figure 4). Hematoxylin counterstaining provided good contrast for visualization of the overall architectural arrangement, cytoplasmic features, and nuclear structures, and permitted excellent general evaluation of the smears. The cytologic and histologic features, including the positive results of Oil Red O staining, led to a final diagnosis of well-differentiated liposarcoma for 3 of the 4 tumors and of pleomorphic liposarcoma for the fourth.

Table 1. Cytologic and histologic diagnoses and Oil Red O staining results for 12 tumors from dogs.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Cytologic Diagnosis</th>
<th>Histologic Diagnosis</th>
<th>Oil Red O Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liposarcoma</td>
<td>Well-differentiated liposarcoma</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Hemangiopericytoma</td>
<td>Hemangiopericytoma</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Liposarcoma</td>
<td>Well-differentiated liposarcoma</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>Sarcoma</td>
<td>Fibrosarcoma</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Poorly-differentiated sarcoma</td>
<td>Undifferentiated sarcoma</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Liposarcoma</td>
<td>Well-differentiated liposarcoma</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>Sarcoma with multinucleated giant cells</td>
<td>Malignant fibrous histiocytoma</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>Hemangiopericytoma</td>
<td>Hemangiopericytoma</td>
<td>Focally positive</td>
</tr>
<tr>
<td>9</td>
<td>Sarcoma</td>
<td>Fibrosarcoma</td>
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</tr>
<tr>
<td>10</td>
<td>Sarcoma</td>
<td>Fibrosarcoma</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>Poorly-differentiated liposarcoma</td>
<td>Pleomorphic liposarcoma</td>
<td>Positive</td>
</tr>
<tr>
<td>12</td>
<td>Hemangiopericytoma</td>
<td>Hemangiopericytoma</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Figure 1. Cytologic smear of a liposarcoma. The neoplastic cells have indistinct cytoplasm filled with round, sharply demarcated vacuoles. May-Grünewald Giemsa, ×100 objective.

Figure 2. Histologic section of a well-differentiated liposarcoma, in which vacuolation of neoplastic cells and fibrous septa are evident. H&E, ×40 objective.
Three tumors were definitively diagnosed as hemangiopericytomas based on both cytologic and histologic examination (Table 1). In cytologic specimens, the cells had exfoliated in 2-dimensional, noncohesive sheets, and in 1 sample a focal perivascular arrangement of cells was seen. Cytologic specimens contained spindle or stellate cells with slightly blue cytoplasm and oval to round nuclei with finely irregular chromatin. All smears contained binucleated and multinucleated cells; in the latter, nuclei were arranged around the cytoplasmic border leading to their designation as “crown cells.” In 2 of the tumors the cytoplasm of the cells contained small vacuoles; in 1 of these tumors, the vacuoles were partially and weakly positive to Oil Red O staining (Figure 5).

Five cases were diagnosed as unclassified sarcomas based on cytologic examination. The cytologic specimens contained either single or aggregated spindle cells, with variable amounts of light or deep blue cytoplasm that frequently was microvacuolated. Large nuclei with clumped chromatin, anisokaryosis, and macronucleoli were observed. All of the smears contained bi- or trinucleated cells, and 1 sample contained multinucleated giant cells. Histologically, 1 tumor was diagnosed as a poorly differentiated sarcoma, with large, plump ovoid or caudate, deeply basophilic cells with a few microvacuoles, irregularly shaped nuclei with clumped chromatin, and frequent atypical mitoses. Three of these tumors were diagnosed histologically as fibrosarcomas. The sarcoma with multinucleated giant cells in cytologic specimens was classified histologically as malignant fibrous histiocytoma. The tumor diagnosed as poorly differentiated sarcoma by cytologic examination was classified histologically as undifferentiated sarcoma. Of the 5 tumors with a cytologic diagnosis of unclassified sarcoma, none reacted positively with Oil Red O stain (Figure

Figure 3. Cytologic smear of a well-differentiated liposarcoma. Note the red-orange color of the cytoplasmic vacuoles, indicative of lipid. Oil Red O with hematoxylin counterstain, ×100 objective.

Figure 4. Cytologic smear of a pleomorphic liposarcoma. Note the positive staining of cytoplasmic microvacuoles. Oil Red O with hematoxylin counterstain, ×100 objective.

Figure 5. Cytologic smear of a hemangiopericytoma. Note the positive staining of small, irregular vacuoles in the cytoplasm. Oil Red O with hematoxylin counterstain, ×100 objective.

Figure 6. Cytologic smear of a fibrosarcoma. The spindle cells are unstained, but the background is focally positive for lipid. Oil Red O with hematoxylin counterstain, ×100 objective.
6), with the exception of a few fat globules in the background of all smears that stained a typical red-orange color.

Discussion

In this series of cases, Oil Red O was used to accurately diagnose liposarcomas in cytologic specimens based on the identification of cytoplasmic lipid. Cells in 1 of 8 other sarcomas also were positive for lipid, however, a diagnosis of hemangioepitcytoma was made in that case based on other cytologic features.

Liposarcoma is a rare tumor in dogs and cats, representing only a small percentage of all animal neoplasms. The tumor may arise in almost any body site and has been found in spleen,12,13 bone,14–16 abdominal organs,17 extradural spinal location,18 liver,19 heart,20 and bone marrow21,22; however, the subcutis of extremities and the trunk is the most common site.22,23 In humans, liposarcomas often are localized to deeper soft tissues, the gluteal region, thigh, lower portion of the extremities, and retroperitoneum. Histologic diagnosis of liposarcoma is made on the basis of light microscopic results, and the neoplasm is subdivided into well-differentiated, myxoid, and pleomorphic types.5 In well-differentiated variants, the neoplasm is characterized by round, spindle, or stellate cells, and vacuolated cytoplasm. Frequent coalescence of the vacuoles gives rise to a single large vacuole that displaces the nucleus at the periphery of the cytoplasm. In pleomorphic or anaplastic types, only a small percentage of cells contain cytoplasmic vacuolation, but the characteristic morphology of deformed nuclear profiles is often observed. The myxoid variant is identified by neoplastic cells that are loosely arranged in mucoid, Alcian blue-positive stroma.5 In the present series of cases, 3 tumors were definitively diagnosed as well-differentiated liposarcoma, and 1 was the pleomorphic type; myxoid variants were not observed. Ultrastructural studies and special stains for neutral fats often are helpful in establishing the lipid content of cytoplasmic vacuoles.5,23

The cytologic diagnosis of liposarcoma in 3 of 4 cases was suspected on the basis of previously published criteria such as large, round, cytoplasmic vacuoles,6–9 presence of lipoblasts,6,8 peripheralization of nuclei,6,8 and perivascular arrangement of cells.6,24–26 Cells in 1 of the 4 cases had minimal evidence for differentiation, with ovoid neoplastic cells, variable numbers of small to medium-sized vacuoles, and nuclear pleomorphism. Our observations based on the cytologic examination of samples with morphologic features suggestive of liposarcoma were supported by clear evidence of positivity of the cytoplasmic vacuolation to Oil Red O, identifying a crucial feature of this type of neoplasm, compared with that of other mesenchymal tumors, which appeared to be weakly positive in only 1 case of hemangioepitcytoma.

Oil Red O stain has been used in human cytopathology.27 Kim and Goldblatt describe the use of Oil Red O staining of air-dried cytologic smears to differentiate malignant fibrous histiocytoma from pleomorphic variants of liposarcoma and rhabdomyosarcoma in humans.28 Hoenerhoff documented Oil Red O-positive staining of liposarcomatous regions in histlogic sections of a multipotential osteosarcoma in a dog.29 To the best of our knowledge, this is the first report about use of Oil Red O in staining cytologic samples from canine neoplasms. Further studies will be necessary to validate the method for making a final cytologic diagnosis of liposarcoma, including criteria to avoid false-positive results and for correctly interpreting the presence of fatty material.

In conclusion, Oil Red O is a simple, inexpensive, and useful stain for demonstrating the presence of fat in cytologic samples, which might be ideal material for confirming a morphologic suspicion of neoplasia originating in adipose tissue. It should be mentioned that we used air-dried specimens that were not treated with alcohol-based solvents. Our results strongly suggest that Oil Red O can assist in the diagnosis of liposarcoma and, in particular, might aid in correct interpretation of the lipid content of the vacuolation observed in cytologic samples. Moreover, a suspicion of liposarcoma in cytologic specimens that is supported by positive Oil Red O staining might be useful preliminary information for selecting the best fixation procedures to be adopted after surgical removal of masses to obtain a more rapid, definitive, histologic diagnosis.

References