Primary Mesenchymal (Nonangiomatous/Nonlymphomatous) Neoplasms Occurring in the Canine Spleen: Anatomic Classification, Immunohistochemistry, and Mitotic Activity Correlated with Patient Survival

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Abstract. Surgical submissions from canine splenectomy cases spanning a 3-year period (1988–1990) were evaluated. Eighty-seven neoplasms of the spleen considered to be of nonangiomatous and nonlymphomatous origin were selected for morphologic classification, mitotic index determination, immunohistochemical analysis, and patient survival determination. In 76/87 cases, patient survival information was available, and the mitotic index was determined in 83/87 cases. Immunohistochemistry for selected antigens (vimentin, desmin, smooth muscle actin, myosin, and factor VIII-related antigen) was performed in 58/87 of the cases. Morphologic classification of these lesions in standard HE preparations yielded the following neoplastic groups: fibrosarcoma (19/87), undifferentiated sarcoma (19/87), leiomyosarcoma (14/87), osteosarcoma (8/87), mesenchymoma (7/87), myxosarcoma (6/87), histiocytic sarcoma (6/87), leiomyoma (3/87), lipoma–myelolipoma (2/87), liposarcoma (2/87), and malignant fibrous histiocytoma (1/87). A lack of distinct morphologic characteristics among many of the neoplasms that were classified as either fibrosarcoma, leiomyosarcoma, or undifferentiated sarcoma contrasted these groups with the relatively unambiguous features that distinguished the other sarcoma groups. Using immunohistochemical staining for muscle-specific antigens (desmin, smooth muscle actin, and myosin), specific staining often overlapped extensively within the neoplastic groups of fibrosarcomas, leiomyosarcomas, and undifferentiated sarcomas, suggesting either ambiguous morphologic findings or the possibility of a common histogenesis from smooth muscle trabeculae or a distinct population of splenic myofibroblasts. The biological behavior of all tumors examined could be placed into three categories of patient survival: (1) benign, noninvasive tumors (leiomyoma, lipoma) with prolonged survival intervals; (2) malignant tumors (fibrosarcoma, undifferentiated sarcoma, leiomyosarcoma, osteosarcoma, myxosarcoma, histiocytic sarcoma, and liposarcoma), showing severely truncated survival (median 4 months with 80–100% mortality after 12 months; and (3) intermediate survival periods (median 12 months with 50% 1 year survival) attributed to a single group of neoplasm, the mesenchymomas. The biological behavior of primary splenic nonangiomatous, nonlymphomatous sarcomas was most closely correlated with observed mitotic index. Splenic neoplasms of this type with a mitotic index < 9 showed significantly (P < 0.0001) longer survival intervals than those with an index > 9. With the exception of osteosarcoma, all anatomically defined tumor groups contained one or more specimens with a mitotic index < 9. The clinical prognosis given for splenic sarcomas should be modified according to the mitotic index as a predictive value for patient survival.

Key words: Canine species; immunohistochemistry; mitotic index; myofibroblast; nonangiomatous; nonlymphomatous; patient survival; sarcoma; spleen.

Much of the effort expended in the pathologic examination of surgically derived canine tissue is applied to identification and classification of neoplastic diseases. The major utility of this effort lies in prognosis or the correlation of anatomic classification with biological behavior of the disease. Under ideal circumstances, the morphologic classification of neoplastic diseases would clearly communicate their biological behavior and speak to their natural history. By implication, this type of classification would then provide integral information important to appropriate therapy. Unfortunately, in many categories of neoplasia, the classification scheme is based principally on microanatomic features, which offer no portent of effect. Access to the kind of specific prognostic and behavioral data necessary for the pathologist to relate useful information to the clinician treating the case is necessarily dependent on evaluation of retrospectively gathered information that correlates anatomic classification and biological behavior with patient survival data.5,4,10,11,14,15,20,24,25 These concepts and observations have been applied to the canine spleen, an organ that is frequently the subject of surgical interven-
tion and pathologic examination due to nodular or diffuse splenomegaly, rupture, and abdominal hemorrhage. Although the biological behavior and anatomic characteristics of neoplastic angiomatous (i.e., hemangiosarcoma) and lymphoid lesions (i.e., lymphosarcoma) are prevalent enough to have been well documented, the same cannot be said for all other primary neoplastic lesions arising in the spleen. Although in a cumulative sense the latter are nearly as prevalent as splenic hemangiosarcoma, their apparent prevalence is diluted by fragmentation into numerous morphologic categories. These so-called “nonangiomatous” and “nonlymphomatous” sarcomas have been described in some detail but the behavioral characteristics of each anatomically designated category lack the necessary numeric data to provide convincing and useful survival information. This retrospective study supplements existing information related to patient survival following splenectomy for a variety of splenic sarcomas. Prognostic factors for splenic sarcoma are evaluated through the use of patient survival information, immunohistochemistry, and evaluation of mitotic indices. Also addressed are the apparent ambiguities inherent in the anatomically derived histogenetic classification as they relate to biological activity and histopathologic appearance of an important class of neoplastic disease in the aging canine patient splenic mesenchymal neoplasms.

Materials and Methods

The histologic slides from a total of 87 cases of splenic neoplasia representing canine splenic lesions diagnosed during a 3-year period were retrieved and reviewed. Among 83/87 cases in which the breed was reported, 30 distinct breeds were represented. The most prevalent breeds were: Golden Retriever (10/83), Labrador Retriever (9/83), German Shepherd (6/83), Cocker Spaniel, Cockapoo, and Poodle (5/83), Sheltie, Schnauzer, and Doberman Pincer (3/83). The remaining 19 breeds were represented by one or two cases each. Female dogs appear to be overrepresented among splenic sarcomas (66%, 53/82). The mean age of dogs in this study was 11.3 years (range 3–16). Except for comparative purposes, angiomatous and lymphoid tumors were excluded from consideration in this study. The study included 82 sarcomas of various classification and five benign lesions designated leiomyoma (3/87) and lipoma (2/87). In 85 of the cases a questionnaire was sent to the original submitting veterinarian. All questionnaires were returned but in 9/87 cases information regarding the animal and its survival interval post-surgically was not available. Thus, detailed survival information was available from 76 cases. Among the cases for which information was available, 55/87 were retrieved from the files after being submitted as formalin-fixed surgical specimens derived from splenectomy. Thirty-two (32/87) of the cases were submitted as whole, fresh, refrigerated spleens for complete gross examination and histopathology as part of an ongoing study into the causes and consequences of splenomegaly and splenectomy in dogs. All of the lesions were examined microscopically and categorized anatomically. This initial classification was reviewed (WLS) and revised independently (WLS) at a later date. All the cases were then reexamined a third and fourth time (WLS) as a part of the mitotic index and immunohistochemical evaluation. Mitotic indices were determined in 83 of the cases by counting the number of mitotic figures in ten randomly selected high-power microscopic fields (500 μm diameter) in 5-μm HE-stained sections. Mitotic indices were divided into four groups or numerical ranges (i.e., 0–9, 10–19, 20–29, and 30+), and the survival data for each group were compared statistically. Three (3/87) of the neoplasms were selected for phosphotungstic acid hematoxylin (PTAH) stains based on specific anatomic features suggesting possible origin from skeletal muscle. Lesions selected for immunohistochemistry (53/87) were: fibrosarcoma 14/19, leiomyosarcoma 11/14, myxosarcoma 6/6, undifferentiated sarcoma 14/19, histiocytic sarcoma 4/6, and osteosarcoma 4/8. Each of the selected cases was evaluated for the presence and staining intensity of vimentin, desmin, factor VIII-related antigen, myosin, and alpha smooth muscle actin. Five-micrometer sections from routinely processed paraffin blocks were mounted on poly-L-lysine-coated glass slides and dried in horizontal racks in a 58–60°C oven. Each section was stained by the avidin–biotin complex (ABC) method using standard reagents (Vector Laboratories, Inc., Burlingame, CA). The sections were deparaffinized in xylene and rehydrated in descending concentrations of ethyl alcohol. Sections subjected to trypsinization (factor VIII) were incubated with 0.1% trypsin and 0.1% CaCl₂ in tris-buffered saline for 20 minutes and rinsed twice in cold phosphate-buffered saline (PBS) 5 minutes each. The sections were quenched at room temperature with 0.3% hydrogen peroxide in methanol for 30 minutes followed by two 5-minute PBS rinses to block endogenous peroxidase activity. Normal serum (10% in PBS) was applied to sections for 20 minutes in a moist chamber. Normal goat serum was used for the polyclonal antibodies (factor VIII and vimentin), and normal horse serum was used for monoclonal antibodies (desmin and alpha smooth muscle actin). The normal serum was drained from the slide and followed directly by incubation with the appropriate primary antibody: 1:25 human desmin, clone 33, Medica, Carlsbad, CA; 1:100 alpha smooth muscle actin, clone 1A4, Biogenex Laboratories, San Ramon, CA; 1:150 bovine vimentin, ICN Immunobiologicals, Costa Mesa, CA; 1:1,000 human Von Willebrand factor–factor VIII-related antigen, Dako Corp., Carpinteria, CA; or 1:1,400 monoclonal anti-skeletal myosin-fast, Biomakor, Rehovot, Israel) in a moist room-temperature chamber for 60 minutes. The slides were washed twice for 5 minutes each with PBS. Appropriate biotinylated antibodies (goat antirabbit or horse antimouse) were then applied for 30 minutes in a room-temperature moist chamber, followed by two PBS 5-minute rinses. ABC was mixed, incubated for 30 minutes, and applied to the sections in a moist room-temperature chamber for 30 minutes followed by two PBS rinses of 5 minutes each. DAB (3,3-diaminobenzidine) (Sigma Chemicals, St. Louis, MO) was applied to slides and observed for color development (usually 5 minutes). Slides were then washed...
in tap water and counterstained with Mayer's hematoxylin, dehydrated, cleared, and coverslipped. Positive and negative control tissues were stained with each batch. Sections of normal splenic tissue were used as positive controls for all immunohistochemical stains used. Negative controls were duplicate sections of the examined tissue and normal spleen on which the primary antibody step was omitted. Immunohistochemistry results were interpreted based on the estimated proportion of neoplastic cells that demonstrated a specific staining reaction. This staining was evaluated subjectively on a scale of 0–4. Negative (0) specimens contained no identifiable specific staining reaction. A score of 1 indicated that up to 15% of cells present in the section demonstrated a specific staining reaction for the particular reagent, 2 = 15–40%, 3 = 40–70%, and 4 = >70%. Statistical analysis of survival data was performed using Kaplan–Meier product limit estimates. Survival functions were statistically compared using the log-rank test.

Results

Morphologic classification of each of the splenic mesenchymal tumors was based on gross and microscopic characteristics in an attempt to create histogenetically homogeneous tumor categories. Although varying widely in cellular morphology, the diagnosis of fibrosarcoma (Fig. 1) was restricted to those tumors (19/87), demonstrating intercellular collagenous matrix with a distribution that could reasonably be attributed to production by neoplastic cells. Collagen was readily demonstrable either via polarization or specific staining. All fibrosarcomas subjected to immunohistochemical staining were vimentin positive (14/14). Desmin was demonstrated in 8 of 14; smooth muscle actin was observed in 7 of 13 stained specimens. There was generally good correlation between the presence of desmin and smooth muscle actin (Table 1). None of the fibrosarcoma specimens (0/14) contained positive cells for either myosin or factor VIII-related antigen. The diagnosis of leiomyosarcoma (14/87) was restricted to those lesions lacking evidence of extracellular matrix formation and were most often composed of elongated spindle-shaped cells containing blunt-ended nuclei (Fig. 2). Considerable anatomic variation among neoplasms was tolerated, but the morphology of the best differentiated areas prevailed in categorizing the lesions. Immunohistochemistry of this class of neoplasm showed a high level of positive reaction for vimentin (11/11). Desmin-positive cells were present in 10/11 and smooth muscle actin was found in 4 of 11 specimens stained. One leiomyosarcoma was positive for desmin staining and negative for smooth muscle actin, while the reverse was true in another case. Among leiomyosarcomas, 3 of 11 showed varying degrees of positivity when stained for factor VIII-related antigen and none were positive for myosin. The category of undifferentiated sarcoma (n = 19) was used for those lesions in which there were no anatomic clues regarding tissue of origin. These tumors demonstrated a high level of cellular pleomorphism, lacked evidence of differentiation in any location, and had no diagnostic cytological features or extracellular matrix (Fig. 3). All of the neoplasms stained in this category (14/19) were positive for vimentin, 12 of 14 were positive for desmin (Fig. 4), and 8 of 14 were positive for smooth muscle actin (Fig. 5). Two lesions were positive for smooth muscle actin that were desmin negative, and 1 of 12 was positive for factor VIII-related antigen. Three (3/19) of these tumors with areas containing strap cells were stained with phosphotungstic and hematoxylin (PTAH) but convincing cross striations could not be demonstrated. These same three lesions were among the 14/19 selected for myosin staining; none were positive. Myxosarcomas (6/87) formed a homogeneous category of neoplasms possessing similar gross and microscopic characteristics (Fig. 6). Grossly, mucinous stringy secretions exuded from the cut surfaces and imparted a similar quality to the fixative solution. Microscopically, a uniformly occurring basophilic mucinous intercellular matrix surrounding spindle or stellar fibroblastic type cells distinguished this group as a distinct morphologic entity. All of these tumors were strongly vimentin positive. Desmin was detected in two of six stained specimens, and smooth muscle actin was present in three of six, while one of the six demonstrated positive staining for factor VIII-related antigen. None of the myxosarcomas showed positive staining for myosin. Histiocytic sarcoma (6/87) as a category was characterized by the lack of intercellular matrix and dissociation of pleomorphic polyhedral-shaped cells. These masses were composed of cells with extreme variability in nuclear size and shape (Fig. 7). The lack of apparent intercellular association and cohesiveness distinguished this group anatomically from the undifferentiated sarcomas. Histiocytic sarcomas were weakly positive for vimentin (3/4). Both desmin and smooth muscle actin were detected in 1/4, and none were positive for either myosin or factor VIII-related antigen. Splenic osteosarcoma (8/87) formed a similarly homogeneous morphologic grouping based on the presence and production of neoplastic osseous matrix with entrapment of individual neoplastic cells (Fig. 8). Both vimentin and smooth muscle actin were observed in three of four tumors so stained. The same four neoplasms were negative for desmin, myosin, and factor VIII-related antigen. Malignant fibrous histiocytoma was the diagnosis in a single neoplasm that showed the peculiar storiform pattern of organization previously described for this entity. Mesenchymoma (7/87) as an anatomic class of lesions was based on the presence of two or more distinct cell types differentiating in the same neoplasm (Fig. 9).
Fig. 1. Splenic fibrosarcoma; dog No. 13. Elongated fusiform cells are present in a collagenous background matrix. This dog was euthanized 2 months post-splenectomy with histologically confirmed hepatic metastasis. HE. Bar = 30 μm.

Fig. 2. Splenic leiomyosarcoma; dog No. 29. Fascicles of parallel-oriented cells with blunt-ended nuclei are closely aligned and lack extracellular matrix. This dog was euthanized immediately following surgery due to histologically confirmed hepatic metastasis. Positive vimentin, desmin, and smooth muscle actin. Negative for factor VIII-related antigen and myosin. HE. Bar = 30 μm.

Fig. 3. Splenic undifferentiated sarcoma; dog No. 47. The neoplasm is composed of pleomorphic cells lacking a recognizable anatomic pattern or intercellular matrix. Multinucleated cells are often present. This dog was euthanized 7 months following splenectomy. Metastatic hepatic lesions were noted intraoperatively. Positive vimentin and desmin. Negative for smooth muscle actin, myosin, and factor VIII-related antigen. HE. Bar = 30 μm.
Among those seen in this study, adipose tissue was a consistent feature. This fat was most often combined with areas of edematous clear matrix with embedded spindle, fusiform, or stellate-shaped cells. Myxomatous, osseous, and/or chondroid matrix was also consistently present. Based on the type of tissue present and the morphology of cells, immunohistochemistry was not performed on this class of tumor. There were no unique or unusual features of the lipomas (2/87) observed in the spleen. They occurred as solitary fatty, soft, pale, circumscribed nodules that sometimes contained aggregates of hematopoietic cells (myelolipoma). Mitotic figures were consistently absent. Liposarcoma (2/87), on the other hand, consisted of pleomorphic, often polygonal cells with cytoplasmic lipid droplets of varying size (Fig. 10). Lesions containing large quantities of adipose tissue were generally excluded from immunohistochemistry staining. The term leiomyoma, in this study, was reserved for those lesions that were discrete, expandable nodular splenic masses made up of well-differentiated myocytes with fibrillar eosinophilic cytoplasm and elongated, blunted-ended nuclei, typical of those found in mature smooth muscle (Fig. 11). These tumors (3/87) all showed a uniform strong positive staining reaction with smooth muscle actin (Fig. 12). In one case stained with both vimentin and desmin, the cells were weakly positive for both. Table 1 provides more concise information regarding type of sarcoma and immunohistochemical staining reaction among the splenic sarcomas that were evaluated. Table 2 provides survival information for all classes of splenic mesenchymal tumors including, for comparative purposes, hemangiosarcoma. In addition, a common non-neoplastic disease resulting in splenomegaly and splenectomy (hyperplastic nodule) was included for survival comparison. Data on patient survival were available for 136 dogs. A survival analysis of the splenic tumors in this table indicated that at least one sarcoma group (mesenchymoma) had a significantly greater survival probability than that of the others (P < 0.05). Upon further analysis excluding mesenchymomas, no further differences between tumor groups were found (P > 0.10). When patients with these other sarcomas were grouped together (65 dogs) and their survival compared to patients with mesenchymomas (7 dogs), the survival functions were significantly different (P < 0.02). Patient age appeared to have little if any influence on survival by tumor type, because group median ages (11 to 13 years) were similar.

Figure 13 shows the survival probabilities for three distinct groups: 116 dogs with all sarcomas (except mesenchymomas), 7 dogs with mesenchymoma, and 13 dogs that underwent splenectomy for hyperplastic nodules. As might be anticipated, dogs with non-neoplastic disease (hyperplastic nodule) showed minimal mortality associated with their condition. Almost all categories of splenic sarcomas (including hemangiosarcoma) were highly fatal in the first year following diagnosis. A single class of neoplasm (mesenchymoma) demonstrated an intermediate level of fatality that was statistically different from the group comprised of all other sarcomas (including hemangiosarcoma) (P < 0.02).

Patients were also divided into four groups based on their numerical ranges of mitotic index (Fig. 14), and their survival was compared. Survival probabilities were marked by differences between groups (P < 0.0001). The 0–9 index group showed the longest postoperative survival. The median survival of patients with neoplasms in the 10–19, 20–29, and 30+ mitotic index categories were only 2 months, 1 month, and 1 month, respectively. Moreover, 80–100% of dogs with splenic neoplasms in the three highest mitotic index categories died or were euthanized within 12 months following splenectomy. Table 3 provides mitotic index data for each morphologic category of splenic sarcoma. Note that with the exception of splenic osteosarcoma, all categories included neoplasm(s) with mitotic indices that fell in the 0–9 index range, suggesting that some patients may have extended survival following splenectomy regardless of anatomic tumor type. On the other hand, a single case of splenic osteosarcoma with a mitotic index of 21 has survived well beyond 12 months. Table 4 contains information on the manner of death and the relationship of the patients' death to the splenectomy diagnosis. The largest portion of dogs with a diagnosis of splenic sarcoma were euthanized. Euthanasia, in virtually all of the cases, was based on the neoplastic diagnosis and attendant poor prognosis. In many of those cases, metastatic lesions were observed at the time of splenectomy or developed and were detected subsequent to surgery. The duration of life was truncated sufficiently as a direct result of the neoplasm so that few dogs had an opportunity to die of competing or unrelated causes.

Discussion

The category of splenic neoplasia considered by this study, "nonangiomatous," "nonlymphomatous" sar-
Fig. 5. Splenic undifferentiated sarcoma; dog No. 50. A typical microscopic field illustrates specific positive staining for alpha smooth muscle actin. Avidin–biotin–peroxidase complex method, Mayer’s hematoxylin counterstain. Bar = 30 µm.

Fig. 6. Splenic myxosarcoma; dog No. 36. Pleomorphic stellate-shaped cells are entrapped in mucinous intercellular matrix. This dog was euthanized 1 month following splenectomy due to unspecified complication of the surgical procedure; metastasis was not documented. HE. Positive vimentin. Negative desmin, smooth muscle actin, factor VIII-related antigen, and myosin. Bar = 30 µm.

Fig. 7. Splenic histiocytic sarcoma; dog No. 68. Histiocytoid cellular morphology, dissociation of cells, and absence of intercellular matrix characterize this type of splenic neoplasm. This dog was euthanized 1 month following surgery with unconfirmed hepatic metastasis. HE. Positive vimentin. Negative desmin, smooth muscle actin, factor VIII-related antigen, and myosin. Bar = 30 µm.


Table 1. Microscopic type of sarcoma and number of tumors showing a positive staining reaction for vimentin, desmin, alpha smooth muscle actin (SMA), myosin, and factor VIII-related antigen in 53 dogs.

<table>
<thead>
<tr>
<th>Microscopic Classification</th>
<th>n</th>
<th>Vimentin</th>
<th>Desmin</th>
<th>SMA</th>
<th>Myosin</th>
<th>Factor VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosarcoma</td>
<td>14</td>
<td>14/14</td>
<td>8/14</td>
<td>7/14</td>
<td>0/14</td>
<td>0/14</td>
</tr>
<tr>
<td>Myxosarcoma</td>
<td>6</td>
<td>6/6</td>
<td>2/6</td>
<td>3/6</td>
<td>0/6</td>
<td>1/6</td>
</tr>
<tr>
<td>Histiocytic sarcoma</td>
<td>4</td>
<td>3/4</td>
<td>2/4</td>
<td>1/4</td>
<td>0/4</td>
<td>1/4</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>11</td>
<td>11/11</td>
<td>10/11</td>
<td>4/11</td>
<td>0/11</td>
<td>3/11</td>
</tr>
<tr>
<td>Undifferentiated sarcoma</td>
<td>14</td>
<td>14/14</td>
<td>12/14</td>
<td>8/14</td>
<td>0/14</td>
<td>0/14</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>4</td>
<td>3/4</td>
<td>0/4</td>
<td>3/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

comas comprised 13% of all splenic lesions resulting in splenomegaly and splenectomy. Moreover, 32% of all canine splenic neoplasms submitted to the laboratory for gross and histologic evaluation were part of this general category of splenic disease. Histologically, these lesions have been represented by confusing and many times ambiguous anatomic structure necessarily resulting in numerous and often meaningless categories. Although some of the morphologic groups possessed characteristics suggesting homogeneity within the group (i.e., osteosarcoma, myxosarcoma, mesenchymoma, lipoma, liposarcoma, and leiomyoma), categorization of some tumors was impossible based on standard morphologic features alone, and these become the "undifferentiated" or, in some cases, histiocytic sarcomas. In turn, these categories necessarily overlapped cytologically to a varying extent with less well-differentiated fibrosarcomas and leiomyosarcomas. In a recent report, an apparently heterogeneous group of splenic neoplasms has been classified as malignant fibrous histiocytoma.

The usefulness of immunohistochemistry in the identification of tissue origins for neoplasms is well established. Because the basic proteins used in these analyses are highly conserved in an evolutionary sense, they can be applied with equal validity in a variety of species, including dogs. In this study the immunohistochemical indicators of tissue origin (i.e., desmin, smooth muscle actin, vimentin, myosin, and factor VIII-related antigen) have been compared directly with the proposed histogenesis of tumors derived from categorization based on anatomic (cytologic) features. This comparison indicates substantial differences between the two systems. The most striking difference is the pervasive presence of myoid tissue components among anatomically classified tumors of nonmyogenic origin. More than half of the fibrosarcomas and undifferentiated sarcomas showed evidence

Table 2. Mean and median survival times (in months) of 136 dogs with a pathologic diagnosis of either splenic hyperplastic nodule, splenic hemangiosarcoma, and nonangiomatous/nonlymphomatous splenic sarcoma. Dogs are considered at risk from time of diagnosis.

<table>
<thead>
<tr>
<th>Pathologic Diagnosis</th>
<th>All Dogs</th>
<th>Dogs Surviving Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Survival Time (SE)</td>
</tr>
<tr>
<td>Hyperplastic nodule</td>
<td>13</td>
<td>27.69 (2.22)</td>
</tr>
<tr>
<td>Mesenchymoma</td>
<td>7</td>
<td>12.14 (3.36)</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>16</td>
<td>2.73 (0.97)</td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>51</td>
<td>4.31 (1.21)</td>
</tr>
<tr>
<td>Histiocytic sarcoma</td>
<td>6</td>
<td>1.51 (0.56)</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>13</td>
<td>3.63 (1.39)</td>
</tr>
<tr>
<td>Liposarcoma</td>
<td>2</td>
<td>0.03 (0.00)</td>
</tr>
<tr>
<td>Myxosarcoma</td>
<td>6</td>
<td>5.50 (2.15)</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>8</td>
<td>2.88 (1.54)</td>
</tr>
<tr>
<td>Undifferentiated sarcoma</td>
<td>14</td>
<td>5.00 (1.16)</td>
</tr>
<tr>
<td>All sarcomas†</td>
<td>65</td>
<td>3.53 (0.55)</td>
</tr>
</tbody>
</table>

*NC = not calculated.
† Excludes hemangiosarcoma and mesenchymoma.

Fig. 8. Splenic osteosarcoma: dog No. 72. Pleomorphic polygonal cells closely associated with irregular spicules of osseous matrix. This dog died 1 month post-splenectomy of unknown causes. HE. Bar = 30 μm.
Fig. 9. Splenic mesenchymoma; dog No. 81. The typical appearance of this neoplasm includes adipose tissue associated with osseous or chondroid matrix. Edematous fibrous or myxoid areas are also often present. This dog died 23 months post-operatively as a result of congestive heart failure. HE. Bar = 30 μm.

Fig. 10. Splenic liposarcoma; dog No. 86. Irregular nuclear profiles in pleomorphic cells containing variable-sized lipid droplets. This dog died in the immediate post-surgical period. HE. Bar = 30 μm.

Fig. 11. Splenic leiomyoma; dog No. 62. Expansile, discrete fascicles of well-differentiated smooth muscle are cytologically indistinguishable from splenic trabeculae. This dog was lost to followup. HE. Bar = 30 μm.

Fig. 12. Splenic leiomyoma; dog No. 62. A typical microscopic field illustrating specific staining for alpha smooth muscle actin. A positively stained arteriole is present in the field for comparison. Avidin–biotin–peroxidase method; Mayer’s hematoxylin counterstain. Bar = 30 μm.
of desmin and smooth muscle actin, suggesting myoid origin. The pervasiveness of muscle-associated cytoskeletal proteins in these tumors may be related to histogenetic derivation directly from smooth muscle tissue of the splenic trabecular system or vascular smooth muscle; however, a possible alternate explanation is suggested by the description and recognition of myofibroblasts as well as immunohistochemically distinct myoid cells in the spleen and lymph node. Fig. 13. Product-limit estimates of survival rates in 136 dogs grouped by splenic tumor type: hyperplastic nodule, mesenchymoma, and other sarcomas.

Ultrastructural myoid components suggesting myogenic origin (thin actin filaments, subplasmalemmal densities, etc.) can be found in a variety of tissues. The myofibroblast, described ultrastructurally, is a cell with anatomic and functional features of both fibroblasts and myocytes and encompasses a heterogeneous collection of cells with subtle morphological differences and varying modes of genesis. These cells are described in a variety of locations as normal components of tissue and have been described both as components of neoplastic proliferations and as neoplasms in their own right (myofibroblastoma, myofibroma, myofibrosarcoma). More recently, the immunohistochemical characteristics of myofibroblasts have been investigated and defined. Varying expression of vimentin, desmin, and muscle actins in these cells have been documented based on the cellular environment and are perhaps defined by cytokines. Only a subset of myofibroblasts (those found in fibromatosis and hypertrophic scars) were found to express desmin. There is also evidence that nonmuscle cells can activate smooth muscle-associated genes, supporting the general concept of myofibroblasts. Some fibroelastic types of cells express desmin, thus suggesting some stromal cells are equipped with muscular elements and actually participate in visceral contraction. Desmin, however, is not associated with all muscle types. In addition to myofibroblasts, desmin, smooth muscle actin, and myosin have been described in a subset of reticular cells found in the spleen and lymph node. This information suggests the possibility that among sarcomas in the canine spleen, a distinction between fibroblastic, myoblastic, and primitive stem cell (reticular/undifferentiated) may be impractical at both the ultrastructural and immunohistochemical level of examination because they all contain one or more of the components of myoid differentiation. In three of our tumors, anatomic characteristics suggested myoid origins with skeletal (striated) muscle differentiation. However, we were unable to find convincing cross striations in specially stained preparations (phosphotungstic acid hematoxylin) similar to those described previously, and myosin stains on these sections were negative. There

Table 3. Type of sarcoma, number of cases examined, mean mitotic index observed, and mitotic index range observed (500-μm diameter field) for each microscopic sarcoma type.

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>n</th>
<th>Mitotic Index</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Myxosarcoma</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>Undifferentiated sarcoma</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Histiocytic sarcoma</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>Mesenchymoma</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Leiomyoma</td>
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<td>0</td>
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<td>Osteosarcoma</td>
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<td>Lipoma</td>
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</tr>
<tr>
<td>Liposarcoma</td>
<td>2</td>
<td>19</td>
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were additional incidences in which anatomic differ-
entiated smooth muscle tumors (leiomyosarcomas)
demonstrated prominent factor VIII staining, espe-
cially in which flattened cellular components served
to as the lining of channels containing blood, thus fur-
ther confusing the classification of splenic tumors based
on traditional morphologic features.

Although insight into the origins of these splenic
tumors may be obtained through the use of immu-
nohistochemistry and other staining methods, these
methods appear to provide little useful information or
correlation regarding the biological behavior of the ne-
oplasms and therefore are marginally applicable for
clinical translation. The categories of splenic sarcomas
seem most diverse when evaluated by morphologic
criteria only. Superimposing immunohistochemistry
on this structure tends to rearrange the neoplasms
within each existing category. If behavioral character-
istics of these sarcomas are evaluated statistically, three
distinct categories emerge: (1) benign neoplasms
(leiomyoma and lipoma), for which post-surgical sur-
vival times are extended; (2) malignant tumors (fibo-
sarcoma, leiomyosarcoma, liposarcoma, osteosarco-
ma, myxosarcoma, histiocytic sarcoma, and undifferen-
tiated sarcomas), for which post-splenectomy
survival is truncated and statistically no different
than survival following splenectomy for hemangiosar-
coma (Table 2); and (3) neoplasms with multiple lines
of tissue differentiation (mesenchymoma), which, al-
though considered malignant, display post-operative
survival characteristics intermediate and statistically
different from either of the other groups (Fig. 13). In
all groups of splenic sarcomas (excluding hemangiosar-
coma—for which information is incomplete) the mi-
totic index is most closely related to the ultimate bi-
ological behavior and therefore appears to be the best
morphologic predictor of adverse consequences. Mit-
totic indices <10 (0-9) are highly correlated (P <
0.0001) with extended life expectancy compared to
those neoplasms with mitotic indices greater than 9.
In the latter category animals were much more likely
to die or to be euthanized as a direct result of the
neoplasm in a shorter post-operative period of time
(Fig. 14). Similar conclusions have been reached for
other canine neoplasms.14,25 Mitotic index is consid-
ered generally important in the overall pathologic eval-
uation of surgically derived neoplastic tissues.4

There is, of course, the risk that the diagnosis and
accompanying poor prognosis becomes a self-fulfilling
prophecy that may adversely affect survival. There is
evidence to suggest widely divergent survival times
within tumor categories as defined here and by oth-
ers.24,25 Although a bleak prognosis is warranted based
on this information, until we can in some way distin-
guish those individual lesions that are likely to deviate
widely from the statistical mean, prognostic pro-
nouncements should contain a cautionary note.

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