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Objective—To identify risk factors associated with survival in dogs with nontonsillar oral squamous cell carcinoma (OSCC) that were and were not treated with curative-intent surgery.

Design—Retrospective case series.

Animals—31 dogs with OSCC.

Procedures—Medical records for dogs with OSCC that were not treated, or were treated with curative-intent surgery only between January 1990 and December 2010 were reviewed. For each dog, data regarding signalment, clinical stage, treatment, tumor recurrence, and survival time were obtained from the medical record, and archived biopsy specimens were evaluated to identify the histologic subtype of the tumor and extent of tumor-associated inflammation (TAI), perineural invasion (PNI), and lymphovascular invasion (LVI).

Results—Risk of death for the 21 dogs with OSCC that were surgically treated was decreased 91.4% (hazard ratio, 0.086; 95% confidence interval, 0.002 to 0.150), compared with the 10 dogs with OSCC that were not treated. The 1-year survival rate was 93.5% and 0% for dogs that were and were not surgically treated, respectively. Risk of death increased significantly with increasing TAI and increasing risk score (combination of TAI, PNI, and LVI). Tumor location, clinical stage, and histologic subtype were not associated with survival time.

Conclusions and Clinical Relevance—Results indicate that the prognosis for dogs with OSCC was excellent following surgical excision of the tumor. Risk of death increased with increasing TAI, and combining TAI, PNI, and LVI into a single risk score may be a useful prognostic indicator for dogs with OSCC. (J Am Vet Med Assoc 2013;243:696–702)

Oral squamous cell carcinoma is an invasive epithelial neoplasm that was first described in dogs over 50 years ago, and diagnostic and treatment options for affected dogs continue to evolve.1–3 Oral squamous cell carcinoma is the second most common malignant oral neoplasia in dogs4,5 and the most prevalent malignant oral neoplasia in humans.6–8 Following treatment, the outcome for dogs with localized nontonsillar OSCC is generally good,5 compared with the outcome following treatment for dogs with tonsillar OSCC, which has a high probability of metastasizing and consequently a poor prognosis.5,9 Study of OSCC in dogs has been proposed as a model for the disease in human patients2; however, differences in OSCC between dogs and humans have been described.3 Further investigation of OSCC in dogs is necessary to identify histologic variations and risk factors associated with tumor recurrence, treatment outcome, and survival.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<td>HR</td>
<td>Hazard ratio</td>
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<td>LVI</td>
<td>Lymphovascular invasion</td>
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<td>MST</td>
<td>Median survival time</td>
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<td>OSCC</td>
<td>Oral squamous cell carcinoma</td>
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<td>PNI</td>
<td>Perineural invasion</td>
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<td>SCC</td>
<td>Squamous cell carcinoma</td>
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<td>TAI</td>
<td>Tumor-associated inflammation</td>
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In dogs, OSCC can be divided into histologic subtypes that are in many but not all aspects similar to OSCC in humans. These subtypes include well-, moderately, and poorly differentiated conventional SCCs as well as more rare subtypes such as papillary and basaloid SCCs and adenosquamous and spindle cell carcinomas.1 In human patients, well-differentiated OSCC are more frequently found in the tongue than elsewhere in the oral cavity, and well- and moderately differentiated tumors are more likely to have TAI than are other histologic subtypes.3 The prognosis for human patients with OSCC is primarily dependent on the clinical stage of disease; however, it is also associated with location and histologic subtype of the tumor, PNI, and LVI. The effect of TAI on prognosis for patients with OSCC is controversial.6,10–12

The 1-year survival rate ranges from 84% to 91% and the metastatic rate ranges from 3% to 36% in dogs.
following surgical resection of OSCCs. The prognosis for dogs with OSCC is generally excellent following radical surgical excision of the tumor via mandibulectomy, maxillectomy, or glossectomy; however, to our knowledge, the effects of histologic subtype, TAI, LVI, and PNI of the OSCC on prognosis have not been evaluated.

The objective of the study reported here was to compare clinical and histologic variables between dogs with OSCC that were treated with curative-intent surgery without adjuvant radiation or chemotherapy and dogs with OSCC that were not treated. Our goal was to identify risk factors that could be used as prognostic indicators for dogs with OSCC. We hypothesized that dogs with poorly differentiated histologic subtypes of OSCC would have less favorable outcomes than dogs with well- or moderately differentiated histologic subtypes of OSCC.

Materials and Methods

Case selection—Medical records and pathology reports for dogs that were admitted to the William R. Pritchard Veterinary Medical Teaching Hospital between January 1990 and December 2010 were searched by the use of the following keywords: canine, oral cavity, and SCC. Dogs with nontonsillar OSCC that were treated with curative-intent surgical excision of the tumor without adjuvant radiation or chemotherapy and dogs in which nontonsillar OSCC was diagnosed but not treated were included in the study. Dogs with nontonsillar OSCC that were administered radiation or chemotherapy or for which complete data were unavailable were excluded from the study.

Medical records review—Age, sex, weight, tumor location, tumor size, regional and distant metastasis, treatment, tumor recurrence, and survival time were recorded for each dog. Tumor location was defined as previously described. Data used to determine the patient's clinical stage of disease were reviewed and included tumor measurement, examination of regional lymph nodes, and 3-view thoracic radiographs. For dogs with which cytologic aspirates or histologic biopsy specimens of the regional lymph nodes were unavailable, the size and consistency of the regional lymph nodes during palpation were recorded. The clinical stage for each dog was determined in accordance with the World Health Organization's TNM system for classification of tumors in domestic animals. Dogs that were surgically treated underwent mandibulectomy, maxillectomy, or glossectomy as described, and surgeons attempted to achieve surgical margins of at least 10 mm during all tumor excisions. The survival time for each dog was calculated as the number of days from histologic diagnosis of OSCC to death. When data to calculate survival time were not available in a dog’s medical record, the owner was contacted to determine whether the dog had died, and if it had, the approximate date of death, whether it died or was euthanized, and whether its death was related to OSCC.

Histologic evaluation and classification of archived OSCC specimens—For each dog, all pathology reports regarding the OSCC were reviewed. At the time of OSCC diagnosis, immunohistochemistry stains (cytokeratin AE1/AE3) were used when the histogenesis was ambiguous (ie, putative epithelial cells with a spindleoid morphology were identified, a frequent finding in spindle cell carcinomas or poorly differentiated OSCCs). Histologic margins were considered narrow when tumor cells were present within 2 mm of the tissue margin and incomplete when tumor cells were seen at the margin of the resected tissue.

Additionally, 5-µm-thick sections were obtained from archived formalin-fixed and paraffin-embedded OSCC tissue specimens as described and histologically evaluated by a board-certified veterinary pathologist (BGM). Each OSCC was categorized by subtype into 1 of 4 groups; group 1 consisted of conventional well-differentiated OSCC, group 2 consisted of conventional moderately differentiated OSCC, group 3 consisted of papillary OSCC, and group 4 consisted of conventional poorly differentiated OSCC, basaloid SCC, and adenosquamous and spindle cell carcinomas. Tumor-associated inflammation and the penetration of tumor cells into lymphatic or blood vessels (LVI) and surrounding nerves (PNI) were also evaluated. Tumor-associated inflammation was subjectively graded as absent (0%), mild (> 0% to < 5%), moderate (≥ 5% to < 40%), or severe (≥ 40%) on the basis of the percentage of inflamed surface area present in the section evaluated.

The nature of the inflammatory infiltrate (eg, suppurative or mononuclear inflammation) was not categorized. Lymphovascular invasion and PNI were dichotomously classified as either present or absent.

Statistical analysis—For comparison purposes, dogs were categorized into 3 age groups (< 2 years, 2 to < 10 years, and ≥ 10 years). A numeric score was assigned to each category for TAI, LVI, and PNI. Absent, mild, moderate, and severe TAI were assigned scores of 0, 1, 2, and 3, respectively. For LVI and PNI, a score of 0 or 1 was assigned when the condition was absent or present, respectively. For each dog, a risk score was calculated by combining the individual scores for TAI, LVI, and PNI, and dogs were categorized into 1 of 4 categories on the basis of risk score (0, 1, 2, and ≥ 3) for comparison purposes.

Pearson χ² tests were used to compare distributions of sex and breed in the study population with those of the hospital population between 1990 and 2010 and to evaluate frequency of OSCC among unordered categorical variables (ie, sex, breed, OSCC subtype, and tumor location). Kruskal-Wallis tests were used to analyze differences in the distribution of non-Gaussian continuous data among ordinal categorical variables (ie, age, clinical stage, and inflammation). The associations of OSCC histologic subtype and completeness of tumor excision with survival time were assessed with a log-rank test. A Fisher exact test was used to evaluate the respective associations between treatment and PNI and LVI. A Kaplan-Meier product-limit method of survival function estimation was used to evaluate the respective associations of age, clinical stage, and risk score with survival time. For the survival analyses, dogs were censored if they had died from causes other than OSCC, were lost to follow-up, or were still alive at the end of the study. Cox proportional hazard regression models were used to calculate HRs and 95% CIs. All analyses...
were performed with statistical software, and values of \( P < 0.05 \) were considered significant.

Results

Animals—Between 1990 and 2010, 84 dogs with OSCC were examined at the veterinary teaching hospital. Thirty-eight dogs were excluded from the study because of incomplete medical records or a lack of follow-up information, and another 15 dogs were excluded because they were administered radiation, chemotherapy, or a combination of treatments. Thus, 31 dogs with OSCC were included in the study. The age and sex distributions for the 31 study dogs did not differ significantly from those of the hospital population. Although Shetland Sheepdog, English Springer Spaniel, and Husky breeds were overrepresented in the study population, a conclusion regarding a breed predisposition for the development of OSCC could not be made because of the small number of each breed represented in the study population.

Of the 31 study dogs, 2 (6%) were < 2 years old, 14 (45%) were between 2 and 10 years old, and 15 (48%) were ≥ 10 years old at the time OSCC was diagnosed, and the mean age for all study dogs was 9 years. The study dogs included 5 (16%) sexually intact males, 12 (39%) castrated males, 2 (6%) sexually intact females, and 12 (39%) spayed females.

OSCC location, subtype, stage, and treatment—Of the 31 OSCCs, 26 (84%) were located on the maxilla or mandible, 4 (13%) were located on the tongue, and 1 (3%) was located on the oral mucosa. Six (19%) and 11 (36%) OSCCs were located on the rostral (ie, extending from first incisor to second premolar) aspect of the maxilla and mandible, respectively, whereas 7 (23%) and 2 (7%) OSCCs were located on the caudal (ie, caudal to the second premolar) aspect of the maxilla and mandible, respectively.

Twenty-four of the 31 (77%) study dogs had conventional OSCC, of which 11 (46%) were classified as well-differentiated (grade 1), 10 (42%) were classified as moderately differentiated (grade 2), and 3 (12.5%) were classified as poorly differentiated (grade 3). The grade 4 category contained 3 dogs with papillary SCC, 2 dogs with adenosquamous carcinoma, 1 dog with basosccoid SCC, and 1 dog with spindle cell carcinoma. Three of 4 tumors located on the tongue were classified as well-differentiated OSCCs. Grade 2, 3, and 4 tumors generally originated from the epithelium of the maxilla or mandible and rarely affected the tongue or other oral surfaces.

Thirteen, 5, and 4 dogs with OSCC had mild, moderate, and severe TAI, respectively, whereas 9 dogs with OSCC did not have TAI. Perineural invasion was detected in 4 dogs and LVI was detected in 1 dog; 1 dog had both PNI and LVI. Of the 31 study dogs, 3 (10%) had a risk score of 0, 10 (32%) had a risk score of 1, 10 (32%) had a risk score of 2, and 8 (26%) had a risk score ≥ 3.

Histologic examination of the mandibular or medial retropharyngeal lymph nodes was performed for 9 of the 31 study dogs, only 2 of which had palpably enlarged lymph nodes at the time of initial physical examination. Histologic examination of lymph node biopsy specimens was performed for 8 study dogs. For the 14 study dogs for which cytologic or histologic examination of the regional lymph nodes was not performed, information recorded in the medical records indicated that the regional lymph nodes were symmetrically soft and small. Thoracic radiography was performed in all 31 study dogs. Nine (29%) dogs had evidence of metastasis; 7 dogs had regional metastasis to the mandibular or medial retropharyngeal lymph nodes, and 3 dogs had distant metastasis to the lungs, with 1 dog having both regional and distant metastatic disease. At the time OSCC was diagnosed, 9 (29%) dogs were classified as having clinical stage 1 disease, 5 (16%) were classified as having clinical stage 2 disease, 13 (42%) were classified as having clinical stage 3 disease, and 4 (13%) were classified as having clinical stage 4 disease.

Treatment of the OSCC was not attempted in 10 of the 31 study dogs. The remaining 21 dogs underwent surgery to remove the OSCC; 7 had a partial maxillectomy, 4 had a partial mandibulectomy, 5 had a bilateral rostral mandibulectomy, 3 had a total mandibulectomy, and 2 had a partial glossectomy. The OSCC could not be completely excised from 2 dogs; one dog was euthanized 420 days after surgery because of OSCC recurrence on the caudal maxilla (the only study dog in which the OSCC recurred) and the other dog (OSCC located on the rostral aspect of the maxilla) was euthanized 7 months after surgery because of a declining quality of life that was not associated with the OSCC. Narrow (tumor cells ≤ 2 mm from the surgical margin) histologic margins were maintained around the excised OSCCs of 4 dogs. The OSCC was located at the rostral aspect of the mandible in 3 of those dogs and at the rostral aspect of the maxilla in 1 dog; however, the OSCC did not recur in any of those dogs. Complete excision of the OSCC with wide surgical (at least 1 cm around grossly diseased tissue) and clean histologic (tumor cells > 2 mm from the surgical margin) margins was achieved in the remaining 15 dogs.

The histologic subtype of the tumor was not significantly associated with tumor location, clinical stage, treatment, extent of TAI, or survival time. Extent of TAI was not significantly associated with histologic subtype, tumor location, or tumor stage. Patient age and tumor location were not significantly associated with the decision of whether to administer treatment; however, dogs with clinical stage 3 or 4 disease were significantly (\( P = 0.001 \)) less likely to be treated (ie, undergo surgical excision of the OSCC) than were dogs with clinical stage 1 or 2 disease.

Survival time—The follow-up period for study dogs ranged from 1 month to 12 years. At the end of the study observation period, only 2 of the 31 (6%) study dogs were still alive. One dog was euthanized because of OSCC recurrence. All 10 dogs that were not treated for the OSCC died or were euthanized because of progression of tumor-associated disease. The remaining 18 study dogs died or were euthanized for reasons unrelated to the OSCC. Necropsy results were available for 7 dogs, of which 6 were not treated and 5 of those had evidence of metastasis; the remaining dog had a unilateral rostral mandibulectomy performed 3 years prior to...
the necropsy, and that dog had no evidence of recurrent or metastatic disease.

The MST for dogs with OSCC that were not treated was 54 days, and the 1-year survival rate was 0%. For dogs with OSCC that underwent surgical excision of the tumor, the MST was 365 days for dogs that were ≥10 years old and was not reached during the observation period for dogs that were <10 years old (Figure 1). The 1-year survival rate for all dogs that were surgically treated was 93.5%; the 1-year survival rate was 100% for dogs <2 years old and 73% for dogs that were 2 to <10 years old. However, age was not associated with survival time for dogs with OSCC that were treated with curative-intent surgery. Likewise, tumor location was not associated with survival time.

Clinical stage of the OSCC was not associated with survival time for dogs that underwent curative-intent surgery. The MST was not achieved during the observation period for dogs with clinical stage 1 disease and was 420, 365, and 50 days for dogs with clinical stage 2, 3, and 4 disease, respectively (Figure 2).

Compared with dogs with OSCC that were not treated, the risk of death was decreased by 91.4% (HR, 0.086; 95% CI, 0.002 to 0.150; \(P<0.001\)) for dogs that underwent curative-intent surgical excision of the tumor. The risk of death for dogs with mild, moderate, or severe TAI was significantly (\(P=0.038\)) greater than that for dogs without TAI (HR, 2.06; 95% CI, 1.04 to 4.07). Also, the risk of death was positively associated with risk score (HR, 1.70; 95% CI, 1.01 to 2.87; \(P=0.046\)). The MST was not achieved during the observation period for dogs with a risk score of 0 or 1, whereas the MST was 365 days for dogs with a risk score of 2 or ≥3 (Figure 3). The 1-year survival rate was 80% for dogs with a risk score ≤1, whereas that for dogs with a risk score ≥2 was 36%.

Discussion

Results of the present study provided additional information regarding risk factors associated with survival time for dogs with nontonsillar OSCC. Similarities and differences in histologic and immunohistochemical properties of OSCCs in dogs and humans have recently been described.\(^{1,2}\) For dogs, the prognosis associated with most oral neoplasias was extremely poor\(^{4,22}\) until the 1980s when surgical excision of tumors with wide surgical margins was introduced.\(^{23-25}\) The results of the present study suggested that the prognosis for dogs with nontonsillar OSCC is very good when the disease is detected and treated early with surgical excision of the tumor.
The risk factors (patient age at diagnosis, tumor location, tumor histologic subtype, clinical stage, TAI, and risk score) evaluated in the present study have been recommended by investigators of other studies as prognostic indicators for dogs and human patients with OSCC. We expected that survival time would decrease as the patient's age at OSCC diagnosis increased. In the present study, age was not significantly associated with survival time; however, the MST and 1-year survival rate for the study dogs did decrease as age increased, findings that were consistent even when dogs that died or were euthanized for reasons unrelated to the OSCC were censored. Although it was not significant, the fact that survival time decreased as patient age increased was most likely associated with factors such as concomitant disease or the owner's or veterinarian's perception of surgical risks, which biased the treatment of older patients to palliative rather than curative measures.

Contrary to results of other studies, tumor location was not associated with survival time for the dogs of the present study; dogs with OSCCs at the rostral or caudal aspects of the maxilla or mandible had similar outcomes as did dogs that had OSCCs on the tongue or other oral mucosal surfaces. In fact, the only study dog that had recurrent tumor growth was a dog on which the OSCC was located on the caudal aspect of the maxilla and could not be completely excised. This finding may suggest that local control of OSCCs located on the caudal aspect of the maxilla or mandible is more difficult than local control of OSCCs located on the rostral aspect of the maxilla or mandible or that the tumors located at the caudal aspects of the oral cavity are more likely to remain undetected until later in the course of the disease than are tumors located at the rostral aspects of the oral cavity. However, conclusions regarding prognosis should not be made on the basis of an unfavorable outcome in 1 dog. Results of other studies indicate that the prognosis for patients with lingual tumors is worse than it is for patients with oral tumors located on the maxilla or mandible, possibly because of the greater proximity of lingual tumors to lymphatic and blood vessels increasing the likelihood of metastasis. Further investigation with a larger study population is warranted to determine whether dogs with lingual OSCC have a poorer prognosis than do dogs with OSCCs located on the maxilla or mandible.

In human patients with OSCC, clinical stage of disease is considered the standard prognostic indicator. Unfortunately, cytologic or histologic examination of regional lymph nodes was not performed for all the dogs of the present study. In addition, aspiration of the medial retropharyngeal and parotid lymph nodes was rarely performed, because of the difficulty accessing those nodes. In another study, results of cytologic examination of lymph node aspirates correlated well with results of histologic examination of lymph node biopsy specimens, whereas palpation of accessible mandibular lymph nodes was an unreliable method for detection of metastasis within those nodes. Because of the retrospective nature of the present study, we chose to use notations in the medical records of the palpation of abnormal lymph nodes as a subjective indication of lymph node metastasis for dogs in which cytologic or histologic evaluation of lymph nodes was not performed. For the dogs of this study, cytologic or histologic evaluation was performed on all lymph nodes that were determined to be abnormal via palpation or diagnostic imaging, and none of the dogs that had palpably normal lymph nodes at the time OSCC was diagnosed were subsequently identified as having metastasis to those lymph nodes. The metastatic rate (29% [9/31]) for the dogs of the present study was similar to that of other studies. Unfortunately, necropsy results were available for only 7 dogs in this study, and it is unknown whether more dogs with metastasis would have been identified had necropsies been performed on the other 22 study dogs that died or were euthanized during the observation period.

In the present study, dogs with OSCC that underwent curative-intent surgery survived significantly longer than did dogs with OSCC that were not treated. However, the clinical stage of disease was associated with whether surgical excision of the tumor was attempted; dogs with more advanced disease (ie, clinical stage 3 or 4) were less likely to undergo surgical excision of the tumor than were dogs at clinical stage 1 or 2, indicating a bias against treating dogs with metastatic disease or large tumors. For study dogs that did undergo surgical excision of the tumor, clinical stage was not significantly associated with survival time, although MST did decrease as the clinical stage of disease increased. This trend toward decreased survival time was likely secondary to an inability to achieve local or regional control via surgical excision of the tumor.

The 1-year survival rate (93.3%) for dogs that underwent surgical excision of the OSCC in this study was similar to those (84% and 91%) determined in other studies for dogs with OSCC following partial maxillectomy or mandibullectomy. Moreover, the survival time for dogs in which the OSCC was incompletely excised or excised with narrow margins was significantly longer than that for dogs with OSCC that were not treated. This suggested that attempted surgical excision of the tumor will benefit the patient even when the tumor is not completely removed. The beneficial effects of partial or complete OSCC removal on patient survival may be the result of a reduction in TAI or improvement of the patient’s quality of life because many OSCCs become ulcerated and interfere with normal oral function.

For the dogs of the present study, the distribution of histologic subtypes of OSCCs was similar to that of another study. In human patients with OSCC, the use of histologic subtype as a prognostic factor is controversial, although it is accepted that patients with well-differentiated tumors tend to have a better prognosis than those with poorly differentiated conventional OSCC and other rarer subtypes such as basaloid OSCC. In the present study, histologic subtype was not associated with survival time; however, because of the small population size, our study may have lacked the power to detect an association between histologic subtype and survival time and further research is warranted.

Similar to histologic subtype, the usefulness of TAI as a prognostic indicator for human patients with
OSCC is controversial; however, it is generally accepted that poorly differentiated tumors have more extensive TAI and behave more aggressively.\textsuperscript{1,6,11,12} In the present study, survival time decreased significantly as extent of TAI increased, despite the fact that extent of TAI was not associated with histologic subtype. Although there may have been too few patients in the present study to draw any conclusions regarding the association between tumor aggressiveness and inflammation, TAI may create a microenvironment around the tumor that affects antitumor immunity, which could result in more aggressive or metastatic tumors.\textsuperscript{10,36–38} Additional research is necessary to elucidate the effects of TAI and OSCC progression in dogs.

In the present study, only 5 of the 31 (16%) study dogs had PNI or LVI. It is possible that PNI or LVI remained undetected during histologic examination of tumor biopsy specimens or that only aggressive tumors develop PNI or LVI. Perineural invasion and LVI are associated with a poor prognosis for human patients with OSCC.\textsuperscript{6,7,27,39} This led to the calculation of a risk score, which incorporated the presence or absence of PNI and LVI along with pattern of tumor invasion. This risk score was significantly associated with local recurrence of OSCC and survival time.\textsuperscript{10,37} In the present study, we altered the calculation of the risk score by replacing pattern of tumor invasion with a measure of the extent of TAI. The risk score as calculated in the present study was significantly associated with survival time. Validation of the risk score as used in this study as a prognostic indicator for dogs with OSCC is necessary; however, we believe this risk score has the potential to provide clinically useful information for clients and clinicians.

The limitations of the present study were those inherent to any retrospective study. The small numbers of certain histologic subtypes of OSCC and dogs in various clinical stages were considered before conclusions were made. Although the results of the present study did not support our hypothesis that histologic subtype of OSCC would be associated with survival time, several clinically relevant risk factors were identified that could be used as prognostic indicators for dogs with OSCC. Survival time for dogs with OSCC that underwent curative-intent excision of the tumor (even if tumor removal was incomplete) was significantly longer than that for dogs with OSCC that did not undergo surgical tumor removal; however, dogs that did not undergo surgical tumor removal were generally at more advanced clinical stages of disease than were dogs that did undergo surgical tumor removal. Also, survival time decreased as extent of TAI and risk score (a combination of TAI, PNI, and LVI) increased. Further validation of the risk score as calculated in this study is necessary to determine its usefulness as a prognostic indicator for metastasis and tumor recurrence following curative-intent surgery in dogs with OSCC.

References


