# Outcome and clinical, pathological, and immunohistochemical factors associated with prognosis for dogs with early-stage anal sac adenocarcinoma treated with surgery alone: 34 cases (2002–2013)

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**OBJECTIVE**
To determine survival time and metastatic rate for dogs with early-stage anal sac adenocarcinoma (ASACA) treated with surgery alone and assess whether specific clinical, pathological, or immunohistochemical factors were predictive of outcome for those dogs.

**DESIGN**
Retrospective case series.

**ANIMALS**
34 dogs with early-stage, nonmetastatic ASACA that were treated with surgery only.

**PROCEDURES**
Medical record databases of 2 referral hospitals were searched to identify dogs examined between 2002 and 2013 that had a diagnosis of nonmetastatic ASACA that was < 3.2 cm at its largest diameter. Only dogs that received surgical treatment alone were included in the study. For each dog, information extracted from the medical record included signalment, clinical and diagnostic test findings, tumor characteristics, and outcome. When available, archived tumor specimens were histologically reviewed and tumor characteristics were described; Ki-67 and E-cadherin expressions were evaluated by use of immunohistochemical methods. Clinical, pathological, and immunohistochemical factors were assessed for associations with survival time and tumor recurrence and metastasis rates.

**RESULTS**
Median survival time was 1,237 days. Seven dogs had tumor recurrence and 9 dogs developed metastatic disease at a median of 354 and 589 days, respectively, after primary tumor removal. Cellular pleomorphism was positively associated with development of metastatic disease. No other factors evaluated were associated with outcome.

**CONCLUSIONS AND CLINICAL RELEVANCE**
Results indicated dogs with early-stage nonmetastatic ASACA generally had a favorable outcome following surgical removal of the primary tumor alone. Routine rectal examination may be a simple and useful method for detection of dogs with early-stage ASACA. (*J Am Vet Med Assoc* 2018;253:84–91)

In dogs, ASACA is an aggressive malignancy with reported metastatic rates that range from 46% to 96%.[1-4] Generally, metastasis initially develops in the lymph nodes that drain the sublumbar region and then progresses cranially over time to the spleen, liver, lungs, and other organs.[1-5] Approximately 25% of dogs with ASACA develop paraneoplastic hypercalcemia, which has a negative effect on prognosis.[6] Results of some studies[6,7] suggest that dogs in which ASACA is diagnosed during the early stages of the disease have a better prognosis than dogs in which the

disease is diagnosed during its later stages; however, those studies included dogs treated with both local and systemic therapies. The metastatic rate and survival time for dogs with early-stage ASACA following surgical treatment alone are unknown, and the value of adjuvant therapy in those dogs is also unclear.

Tumor size is associated with outcome for dogs with ASACA.[6,7] In 1 study,[6] the median survival time was 584 days for dogs with tumors < 10 cm², compared with 292 days for dogs with tumors ≥ 10 cm²; however, dogs with metastatic disease at the time of surgery were not excluded from that analysis, and treatment regimens varied among dogs. Investigators of another study[7] also reported that tumor size was negatively associated with survival time and advocat-

**ABBREVIATIONS**

| | |
|---|---|
| ASACA | Anal sac adenocarcinoma |
| TTP | Time to progression |

ed use of a staging system in which a cutoff of 2.5 cm in diameter for a primary tumor was a central determinant in a patient management algorithm.

The effect of lymph node metastasis on prognosis for dogs with ASACA is less clear. In 1 study,[6] the median survival time did not differ between dogs with and without iliac lymphadenopathy, but those findings were likely confounded by the fact that dogs with lymphadenopathy were more likely to receive chemotherapy than dogs without lymphadenopathy. In another study,[7] lymph node metastasis had a significant negative effect on survival time, and the investigators of that study proposed a staging system in which nodal metastasis was categorized as absent, present with lymph nodes < 4.5 cm in diameter, or present with lymph nodes > 4.5 cm in diameter. In that study,[7] the median survival time for dogs with primary tumors < 2.5 cm in diameter and no lymph node metastasis was 1,205 days. In yet another study,[8] there was a significant negative association between lymph node metastasis and outcome for dogs with ASACA. However, all 3 studies[6-8] were limited by the fact that treatment regimens were not standardized and varied among individual dogs.

Histologic and immunohistochemical characteristics have also been evaluated as prognostic indicators of tumor behavior and outcome in dogs with ASACA. In 1 study,[9] the risk of ASACA-related death was greater for dogs with tumors with a solid or partly solid histologic pattern than for dogs with tumors with rosette or tubular-type patterns. Ki-67, a nuclear marker of cellular proliferation, was identified in ASACA tumor cells for a proportion of the dogs of that study,[9] but its association with clinical or outcome data was not evaluated. In another study,[10] abnormally decreased expression of E-cadherin, a transmembrane protein that mediates cell-cell and cell-matrix interactions, was associated with a poor prognosis for dogs with various stages of ASACA that received various treatments. To our knowledge, the relationship between specific cellular proliferation indices and the clinical behavior or outcome for dogs with ASACA has not been evaluated.

Although previous studies[6-10] have described outcome and prognostic factors for dogs with ASACA, none have controlled for stage of the disease at the time of diagnosis or treatment. The purpose of the study reported here was to determine the survival time and tumor recurrence and metastatic rates for dogs with early-stage ASACA that were treated with surgery alone and assess whether specific clinical, pathological, or immunohistochemical factors were predictive of outcome for that population.

## Materials and Methods

### Case selection criteria

The electronic medical record databases of the Veterinary Medical Teaching Hospital at the University of California-Davis and Centre Vétérinaire Rive-Sud in Québec, Canada, were searched to identify dogs examined between 2002 and 2013 that had a histologically confirmed diagnosis of ASACA with a primary tumor < 3.2 cm at its largest diameter (equivalent to a tumor area of < 10 cm²) and that underwent curative-intent surgery to remove the tumor with no additional treatment. Dogs included in the study also had to have no evidence of metastasis detected during staging tests. Dogs that received additional treatment of any kind after a documented recurrence of ASACA or development of metastatic disease were included in the study. Dogs that had bilateral ASACA; that underwent radiation therapy, chemotherapy, or targeted therapy in the immediate postoperative period; or that had insufficient follow-up information available for assessment of postoperative outcome were excluded from the study.

### Medical records review

For each dog enrolled in the study, data extracted from the medical record included signalment, tumor size and location, staging test results, presence or absence of hypercalcemia, and outcome, including information regarding local recurrence, metastasis, death, and cause of death when available. Referring veterinarians and clients were contacted as necessary to collect follow-up information.

### Histologic and immunohistochemical review of tumor specimens

Available archived tumor specimens that were prepared with H&E stain for histologic examination were reviewed by 2 veterinary pathologists (CAN and EEBL). For each tumor, the predominant (> 50% of the tumor) histologic pattern was characterized as solid, rosette, or tubular. The extent of cellular pleomorphism (ie, variability in cell [anisocytosis] or nuclear [anisokaryosis] size) was categorized as mild (< 25% of cells with anisocytosis or anisokaryosis), moderate (25% to 75% of cells with anisocytosis or anisokaryosis), or marked (> 75% of cells with anisocytosis or anisokaryosis). The number of mitotic figures for ten 40X fields (ie, 10 hpf) was evaluated by each pathologist, and the mean was calculated and used for analysis unless the counts for the 2 pathologists differed by > 5, in which case a consensus count was performed. The extent of necrosis within the tumor was categorized as < 10%, 10% to 25%, > 25% to 50%, or > 50%. The extent of mineralization within the tumor was categorized as 0% (absent), 1% to < 10%, 10% to < 25%, 25% to < 50%, or ≥ 50%. Desmoplasia was categorized as minimal (sparse within the tumor), mild (occasional to frequent within the tumor), moderate (within the tumor and adjacent connective tissue), or severe (within the tumor and all adjacent connective tissues). Intratumoral inflammation was categorized as minimal (rare small clusters or scattered leukocytes), mild (occasional small clusters or scattered leukocytes), moderate (frequent small clusters or scattered leukocytes), or severe (frequent large clusters to sheets of leuko-

cytes). Tumor margins were classified on the basis of definitions adapted from those used for canine soft tissue sarcomas; specific categories included incomplete (neoplastic cells were continuous with at least 1 surgical margin in any plane), narrow (distance between surgically created tissue edge and neoplastic cells was < 3 mm, or surgical margins did not contain normal tissue outside the pseudocapsule), or complete (distance between surgically created tissue edge and neoplastic cells was ≥ 3 mm). Vascular invasion by the tumor was characterized as described.[11]

When available, paraffin-embedded tumor specimens were sliced into 4-μm-thick sections and prepared with immunohistochemical staining methods for detection of Ki-67 and E-cadherin in a manner similar to those previously described,[10,12] except the antibody dilutions were adjusted. Briefly, heat-induced epitope retrieval by means of a retrieval solution[a] was followed by application of either monoclonal mouse anti-human Ki-67 antibody[b] diluted 1:100 in PBS solution or monoclonal mouse anti–E-cadherin antibody[c] diluted 1:500 in PBS solution. Then, a detection system[d] was applied to each specimen, and visualization of the protein of interest was achieved by application of a red substrate kit[e] in accordance with the manufacturer's instructions. Sections were counterstained with Mayer hematoxylin. Internal positive controls were lymphocytic aggregates for Ki-67 and anal sac mucous membrane epithelium for E-cadherin. Negative control slides were prepared in the same manner, except PBS solution was used in place of the primary antibody.

All immunohistochemically stained slides were reviewed by 2 veterinary pathologists (CNA and EEBL). E-cadherin localization was categorized as membranous, cytoplasmic, or nuclear. The distribution or percentage of cells that were immunoreactive for E-cadherin was categorized as < 25%, 25% to 75%, or > 75%. E-cadherin intensity was categorized as light (stippled immunoreactivity), moderate (25% to 50% circumferential membrane immunoreactivity), or strong (> 50% circumferential membrane immunoreactivity). Ki-67 immunoreactivity was evaluated in areas where the staining was most evident or dense and was determined by calculation of the mean for 3 nonconsecutive hpf in which 100 consecutive cells were counted.

## Statistical analysis

Time to local recurrence was defined as the number of days between surgery and documented tumor recurrence in the perianal region. Time to metastasis was defined as the number of days between surgery and diagnosis of metastases at any location. Time to progression was defined as the number of days between surgery and diagnosis of tumor recurrence or metastasis. The TTP was censored for dogs that were alive and did not have tumor recurrence or metastasis at the time of data collection and for dogs that died without tumor recurrence or metastasis. Overall survival time was defined as the number of days between surgery and death, regardless of cause. Overall

survival time was censored for dogs that were alive at the time of data collection or lost to follow-up.

The Kaplan-Meier method was used to estimate the median overall survival time and TTP. The log-rank test was used to assess the association between categorical variables (surgical margins, tumor size, histologic pattern, mitotic index, cellular pleomorphism, vascular invasion, and E-cadherin and Ki-67 immunoreactivity) and survival time. Fisher exact tests were used to assess the respective associations between tumor recurrence or metastasis and categorical variables. Only dogs for which tumor specimens were available for review were included in analyses involving tumor margins or other histologic or immunohistochemical characteristics. For statistical analyses, histologic pattern results were dichotomized as any component of solid architecture versus no solid architecture on the basis of findings of Suzuki et al.[9] Mitotic index results were also dichotomized with the median number of mitotic figures identified/10 hpf used as the breakpoint. The extent of E-cadherin immunoreactivity was categorized as > or ≤ 75% as suggested by other investigators.[10] The extent of Ki-67 immunoreactivity was dichotomized with the median percentage of positive cells used as the breakpoint. All statistical analyses were performed with commercial software,[f] and values of $P <$ 0.05 were considered significant.

## Results

### Dogs

Thirty-four dogs met the inclusion criteria and were included in the study. The study population consisted of 15 spayed females and 19 neutered males with a mean age of 10.5 years (range, 7.2 to 14.8 years). There were 8 mixed-breed dogs, 4 Labrador Retrievers, 3 German Shepherd Dogs, and 19 dogs of other breeds, each of which was represented by ≤ 2 dogs.

### Diagnostic findings

Median tumor diameter was 1.0 cm (range, 0.3 to 3.0 cm). Among the 34 dogs, the ASACA was located in the right anal sac for 22 and the left anal sac for 12. A CBC and serum biochemical profile were performed for all dogs. Two dogs had hypercalcemia at the time of ASACA diagnosis. One of those dogs had a tumor with a diameter of 2.7 cm, and the hypercalcemia resolved after tumor removal. The other dog had a tumor with a diameter of 1.0 cm, and the hypercalcemia did not resolve after removal of the ASACA. That dog was subsequently determined to have primary hyperparathyroidism on the basis of parathyroid hormone and parathyroid hormone–related protein concentrations; the hypercalcemia resolved after removal of a parathyroid adenoma. Thoracic radiography and abdominal ultrasonography (staging diagnostic tests) were performed during the perioperative period for 33 of the 34 dogs, and no evidence of metastatic disease was detected for any of those dogs. The dog that did not undergo staging diagnostic testing at the time of ASACA resection was alive and apparently free of disease at the time of data collection 1,644 days after surgery. Five dogs

also had CT scans performed of the caudal portion of the abdomen including the anal region prior to ASACA resection, and no evidence of metastatic disease was detected on the CT scans of those dogs.

## Histologic and immunohistochemical findings

Tumor specimens for only 20 of the 34 study dogs were available for histologic and immunohistochemical review **(Table 1)**. On the basis of the original histologic reports obtained from the medical records, the excised tumor margins were categorized as complete for 8 dogs, narrow for 13 dogs, and incomplete for 9 dogs; tumor margins were not specified for the remaining 4 dogs. Of the 13 dogs with narrow tumor margins, 10 had tumor margins < 1 mm, and the remaining 3 did not have margin measurements reported. On the basis of the tu-

mor margin categories defined for the present study, 12 of the 20 tumor specimens reviewed had incomplete margins (neoplastic cells were continuous with at least 1 surgical margin in any plane), and the remaining 8 tumor specimens had narrow margins (distance between surgically created tissue edge and neoplastic cells was < 3 mm, or surgical margins did not contain normal tissue outside the pseudocapsule). Interpretation of tumor margins during review for this study was the same as that of the original histologic report for 9 dogs and differed from that of the original histologic report for 8; tumor margins were not specified in the original histologic reports for the remaining 3 dogs. Among the 8 dogs for which the tumor margin interpretations differed between the original report and present study review, 3 had tumor margins that were reclassified from narrow to incomplete, 2 had tumor margins that were reclas-

**Table 1**—Histologic and immunohistochemical characteristics for early-stage nonmetastatic ASACAs resected from 20 dogs that were examined and treated at 2 veterinary referral hospitals between 2002 and 2013.

| Variable | Category | No. (%) of dogs | P value* |
|---|---|---|---|
| Histologic pattern for > 50% of the tumor | Solid | 11 (55) | 0.39 |
| | Rosette | 8 (40) | |
| | Tubular | 1 (5) | |
| Extent of cellular pleomorphism | Mild (< 25% of cells with anisocytosis or anisokaryosis) | 16 (80) | 0.85 |
| | Moderate (25% to 75% of cells with anisocytosis or anisokaryosis) | 4 (20) | |
| | Severe (> 75% of cells with anisocytosis or anisokaryosis) | 0 (0) | |
| Mitotic index (No. of mitotic figures/10 hpf) | < 11.25 | 10 (50) | 0.56 |
| | ≥ 11.25 | 10 (50) | |
| Extent of necrosis in tumor | < 10% | 18 (90) | — |
| | 10% to 25% | 2 (10) | |
| | > 25% to 50% | 0 (0) | |
| Extent of mineralization in tumor | 0% (absent) | 19 (95) | — |
| | 1% to < 10% | 1 (5) | |
| | ≥ 10% | 0 (0) | |
| Desmoplasia | Minimal (sparse within the tumor) | 2 (10) | 0.62 |
| | Mild (occasional to frequent within the tumor) | 12 (60) | |
| | Moderate (within the tumor and adjacent connective tissue) | 5 (25) | |
| | Severe (within the tumor and all adjacent connective tissues) | 1 (5) | |
| Intratumoral inflammation | Minimal (rare small clusters or scattered leukocytes) | 3 (15) | 0.56 |
| | Mild (occasional small clusters or scattered leukocytes) | 8 (40) | |
| | Moderate (frequent small clusters or scattered leukocytes) | 8 (40) | |
| | Severe (frequent large clusters to sheets of leukocytes) | 1 (5) | |
| Histologic tumor margins | Incomplete (neoplastic cells were continuous with at least 1 surgical margin in any plane) | 12 (60) | 0.21 |
| | Narrow (distance between surgically created tissue edge and neoplastic cells was < 3 mm, or surgical margins did not contain normal tissue outside the pseudocapsule) | 8 (40) | |
| | Complete (distance between surgically created tissue edge and neoplastic cells was > 3 mm) | 0 (0) | |
| Vascular invasion by tumor | Absent | 17 (85) | 0.84 |
| | Present | 3 (15) | |
| E-cadherin immunoreactivity (% of positive cells) | < 25% | 3 (15) | 0.14 |
| | 25% to 75% | 5 (25) | |
| | > 75% | 12 (60) | |
| Ki-67 immunoreactivity (% of positive cells) | < 25% | 10 (50) | 0.28 |
| | ≥ 25% | 10 (50) | |

To be included in the study, dogs had to have a histologically confirmed diagnosis of ASACA with a primary tumor < 3.2 cm at its largest diameter (equivalent to a tumor area of < 10 cm$^2$), undergone curative-intent surgery to remove the tumor with no ancillary perioperative treatment, and have no evidence of metastasis detected during staging tests. Dogs that received additional treatment of any kind after a documented recurrence of ASACA or development of metastatic disease were included in the study. Dogs that had bilateral ASACA; that underwent radiation therapy, chemotherapy, or targeted therapy in the immediate postoperative period; or had insufficient follow-up information available for assessment of postoperative outcome were excluded from the study. Thirty-four dogs were enrolled in the study, but tumor specimens from only 20 dogs were available for histologic and immunohistochemical examination and review.

*P value for log-rank test used to compare survival time among categories.

— = Not calculated.

sified from complete to incomplete, 2 had tumor margins that were reclassified from incomplete to narrow, and 1 had tumor margins that were reclassified from complete to narrow. Tumor specimens were available for review for only 3 of the 8 dogs for which the tumor margins were originally classified as complete, and the tumor margins for 2 of those dogs were reclassified as incomplete after review.

The median mitotic index was 11.25 mitotic figures/10 hpf (range, 4 to 30 mitotic figures/10 hpf; mean, 12.7 mitotic figures/10 hpf). E-cadherin was localized in the anal sac mucous membrane epithelium in all 20 tumors that were available for review, and E-cadherin expression was classified as strong or moderate in areas of immunoreactivity for 16 and 4 tumors, respectively. Ki-67 expression varied markedly within individual tumors, and care was taken to ensure that regions with the greatest density of immunoreactive cells were used for the Ki-67 counts. The median percentage of Ki-67–immunoreactive cells in those areas was 25% (range, 13% to 48%; mean, 26%).

## Postoperative follow-up and survival time

The postoperative reevaluation protocol was not standardized for the study population. However, most dogs were reevaluated at 3-month intervals, and those evaluations typically included a physical examination, thoracic radiography, and abdominal ultrasonography.

Seven of the 34 (21%) dogs had local recurrence of ASACA, and the median time to recurrence was 354 days (range, 117 to 947 days). The surgical tumor margins were classified as incomplete for 3 of those dogs and narrow for the remaining 4 dogs. Nine (26%) dogs developed metastasis to regional lymph nodes, and the median time to metastasis was 589 days (range, 99 to 1,147 days). Four dogs had both local tumor recurrence and metastasis. One of the 9 dogs that developed metastatic disease was hypercalcemic at the time metastasis was identified but did not have hypercalcemia at the time ASACA was originally diagnosed. Four dogs (1 with local tumor recurrence, 1 with metastatic disease, and 2 with both local tumor recurrence and metastasis) had additional surgical treatment after progressive disease was diagnosed. One of those 4 dogs also received radiation therapy, and another dog also received mitoxantrone chemotherapy. One dog with metastatic disease was treated with carboplatin chemotherapy only, and 1 dog with tumor recurrence and metastasis was treated with masitinib only. Five dogs (1 with local tumor recurrence, 3 with metastatic disease, and 1 with both local tumor recurrence and metastasis) received no further antineoplastic treatment.

At the time of data collection, 20 dogs had died, 13 were alive, and 1 was lost to follow-up at 1,194 days after surgery. Of the 20 dogs that died, 15 died of causes related to ASACA or of unknown causes, and 5 died of causes unrelated to ASACA. A necropsy was performed on 3 of those dogs, and ASACA was

not detected in any histologically examined tissues from those dogs. The cause of death was hemangiosarcoma for 1 dog, hypertensive vasculopathy for 1 dog, and subcutaneous necrotic sarcoma for 1 dog. For 2 dogs that died but did not undergo necropsy, results of antemortem diagnostic testing did not yield any evidence of ASACA recurrence or metastasis, and the cause of death was reported as congestive heart failure for 1 and chronic renal failure for the other. For all 34 dogs, the median TTP was 851 days (2.3 years) and median overall survival time was 1,237 days (3.4 years; **Figure 1**).
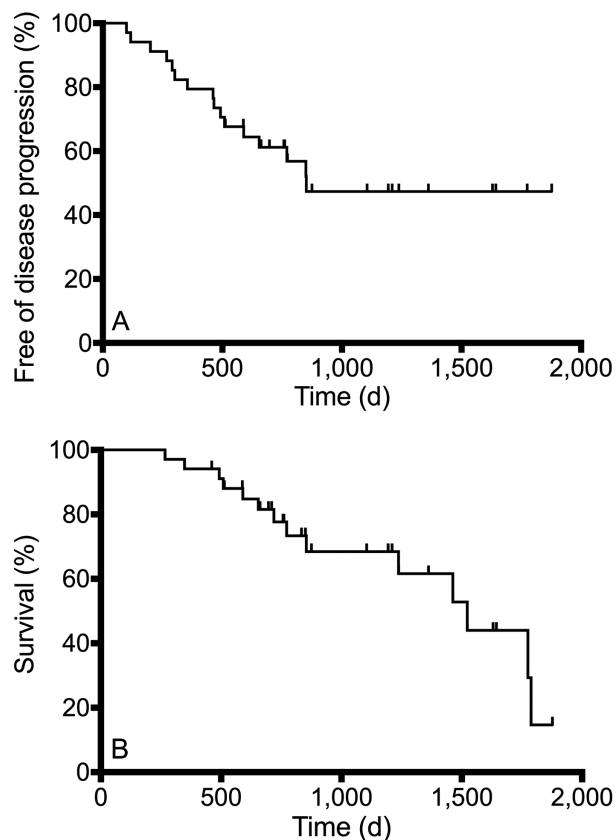


**Figure 1**—Kaplan-Meier curves for TTP (A) and survival time (B) for 34 dogs with early-stage ASACA that were treated with surgery only at 2 veterinary referral hospitals between 2002 and 2013. To be included in the study, dogs had to have a histologically confirmed diagnosis of ASACA with a primary tumor < 3.2 cm at its largest diameter (equivalent to a tumor area of < 10 cm²), have undergone curative-intent surgery to remove the tumor with no ancillary perioperative treatment, and have no evidence of metastasis detected during staging tests. Dogs that received additional treatment of any kind after a documented recurrence of ASACA or development of metastatic disease were included in the study. Dogs that had bilateral ASACA; that underwent radiation therapy, chemotherapy, or targeted therapy in the immediate postoperative period; or that had insufficient follow-up information available for assessment of postoperative outcome were excluded from the study. Vertical tick marks represent censored dogs. Dogs that were alive and did not have evidence of tumor recurrence or metastasis at the time of data collection were censored for the TPP analysis. Dogs that were alive at the time of data collection or lost to follow-up were censored for the survival time analysis.

Extent of cellular pleomorphism was significantly ($P = 0.03$) associated with the development of metastasis. Only 2 of the 16 dogs with mild cellular pleomorphism developed metastatic disease, whereas 3 of 4 dogs with moderate cellular pleomorphism developed metastatic disease. Tumor size, histologic tumor margins, histologic pattern, vascular invasion, mitotic index, E-cadherin localization, and Ki-67 proportion were not significantly associated with survival time, tumor recurrence, or metastasis.

## Discussion

Results of the present study suggested that dogs with early-stage ASACA treated by surgery alone may be at lower risk for metastatic disease than previously reported. Only 9 of the 34 (26%) dogs of the present study developed metastatic disease, whereas the rate of metastatic disease in dogs with ASACA reported in other studies[1-4] ranges from 46% to 96%. However, case selection criteria ensured that only dogs with early-stage nonmetastatic ASACA were evaluated in the present study, whereas dogs of varying stages of the disease were included in those other studies.[1-4] Therefore, although tumor size was not associated with the risk of metastatic disease for the dogs of the present study, it is likely that primary tumor size and stage are risk factors for metastatic disease and are negatively associated with survival time. Many dogs of the present study survived for at least 4 years after surgical removal of the ASACA without any additional treatment. That finding, in conjunction with the subsequent fairly low incidence of metastatic disease, suggested that surgical resection of the primary tumor alone can be an effective treatment for dogs with early-stage nonmetastatic ASACA. Thus, we propose that adjunctive chemotherapy may not be indicated for most dogs with early-stage ASACA. Additionally, the results of this study appeared to suggest that rectal examination should be included in routine physical examinations and may be useful as a simple screening test to detect early-stage ASACA, especially in older dogs. It is important to note that, because of the retrospective nature of this study, some dogs did not undergo reevaluation for tumor recurrence and metastasis as recommended, which might have resulted in underestimation of the metastatic disease rate or overestimation of the TTP for this study population.

Only 7 of the 34 (21%) dogs of the present study had local recurrence of ASACA, despite the fact that surgical resection resulted in incomplete or narrow margins for many tumors. This suggested that local recurrence of ASACA is uncommon in dogs with early-stage disease that are treated with surgery alone. The tumor margins were classified as incomplete or narrow in the original histologic report for 22 of the 30 (73%) dogs of this study for which that information was available. The tumor margins were classified as incomplete or narrow on the basis of the definitions used for this study for all 20 dogs that had tumor specimens available for review. The tumor

margin classification on the original histologic report differed from that determined during this study for 8 dogs, and those discrepancies were likely caused, in part, by variation in the terminology used to describe the margins and underscored the importance of defining terms such as narrow, close, or incomplete when tumor margins are reported. For several dogs, the original histologic report did not specify or define the terms narrow or incomplete. Because guidelines have not been established for the definition of ASACA margins, the criteria used to define the tumor margin categories in this study were adapted from criteria commonly used to define the margins of soft tissue sarcomas.[11] Current consensus guidelines[13] recommend that the distance between neoplastic cells and the surgical tumor margin be reported as well as the type of tissue that comprises the surgical margins because some tissues (eg, fascial planes) provide variable barriers against neoplastic cell invasion. Surgical margins for tumors of the anal sac region are limited by the numerous critical structures adjacent to that area and can rarely exceed 3 mm. Nevertheless, the fairly low rate of tumor recurrence for the dogs of this study suggested that narrow resection of small ASACAs may provide sufficient treatment for most dogs with early-stage disease. Further investigation is necessary to determine optimal surgical margins for ASACAs and better elucidate the role of radiation therapy for tumors with incomplete surgical margins.

Only 1 dog of the present study had hypercalcemia of malignancy at the time of ASACA diagnosis. That dog had a primary tumor that was 2.7 cm in diameter, which was fairly large relative to the tumors of the other dogs in the study population. The hypercalcemia in that dog resolved following tumor removal, which suggested that it was hypercalcemia of malignancy. Results of other studies[3,6,7] indicate that approximately 27% of dogs with ASACA develop hypercalcemia of malignancy, and the prognosis for those dogs is poorer than that for dogs with ASACA without hypercalcemia. The low prevalence of dogs with hypercalcemia in the present study might have been associated with the fact that most dogs had a fairly low tumor burden, resulting in dilution of any potential effects of parathyroid hormone–related protein. It might also indicate that small early-stage ASACAs tend not to produce parathyroid hormone–related protein. It is also important to note that 1 dog of the present study was normocalcemic at the time of ASACA diagnosis but was hypercalcemic when metastasis to regional lymph nodes was subsequently detected. That finding suggested that the hypercalcemia status of dogs with ASACA can change over the course of the disease. Further research into the association between tumor stage and hypercalcemia is warranted.

The pathological characteristics were similar for many of the ASACA specimens reviewed in the present study. Vascular invasion by tumors was uncommon (3/17). In general, the extent of cellular pleomorphism, necrosis, and mineralization was uniform across tumors, whereas the histologic pattern, mitot-

ic index, and extent of inflammation varied among tumors. Although the sample size was small, there was a positive association between cellular pleomorphism and development of metastatic disease. It is reasonable to believe that tumors with aggressive characteristics such as cellular pleomorphism are likely to metastasize, but further research involving a more clinically diverse population of dogs with anal sac tumors is necessary to confirm or refute that.

E-cadherin mediates cell-cell and cell-matrix interactions, and E-cadherin expression is negatively associated with the malignant phenotype and metastatic potential of several types of cancers in human and veterinary medicine.[14-16] In another study[10] of dogs with ASACA, abnormally decreased E-cadherin expression was associated with a poor outcome. For the dogs of the present study, E-cadherin expression was not significantly associated with patient outcome, and E-cadherin was strictly localized to the anal sac mucous membrane epithelium and was not observed in any nuclei. There are several plausible explanations for the discrepancies between the E-cadherin expression results of that other study[10] and the present study. Primarily, the E-cadherin dilution (1:500) and isotype ($IgG2_{aK}$) used for the immunohistochemical staining procedure in the present study differed from the E-cadherin dilution (1:100) and isotype ($IgG1_K$) used in that study[10] and may have affected the number and percentage of immunoreactive cells detected. Differences among E-cadherin immunohistochemical staining protocols are clinically relevant because they can affect the reproducibility of results in a diagnostic setting. Also, the sample size of the other study[10] (n = 36) was 1.8 times that of the present study (20), which might have provided the investigators of that study sufficient power to detect differences in E-cadherin expression that we could not. Finally, the dogs enrolled in the present study were limited to those with early-stage nonmetastatic ASACA, whereas those enrolled in that study[10] were at various stages of the disease, and E-cadherin expression may be more variable in advanced-stage or metastatic tumors than in early-stage tumors.

In some types of cancers, high cellular proliferation is associated with aggressive behavior, high metastatic rates, or poor patient outcome.[17-20] Cellular proliferation can be assessed in several ways, such as identification of mitotic figures and calculation of a mitotic index or by immunohistochemical staining. Results of another study[9] involving 69 dogs with ASACA indicate that Ki-67 was expressed in a smaller proportion of tumor cells than proliferation cell nuclear antigen, but the investigators of that study did not attempt to evaluate the association between Ki-67 or proliferation cell nuclear antigen expression and clinical or outcome data or tumor recurrence and metastasis rates. In the present study, Ki-67 expression was not significantly associated with survival time, tumor recurrence, or metastasis; however, only a small number (n = 20) of tumor specimens were available for immunohistochemical staining, which

likely limited the power for detecting significant associations. Further investigation is necessary to determine the potential prognostic value of proliferation indices in a larger and more diverse population of dogs with ASACA than that evaluated in this study.

In the present study, dogs with early-stage nonmetastatic ASACA that underwent curative-intent surgery alone generally had a favorable outcome. Thus, we propose that adjunctive chemotherapy or other ancillary treatments may not be indicated for most dogs with early-stage ASACA. However, a subset of dogs had tumor recurrence or metastasis following surgical resection of the primary tumor, which indicated that routine postoperative monitoring is prudent. Extent of cellular pleomorphism was significantly associated with the development of metastasis for the dogs of this study; therefore, that feature may be a useful component for inclusion in reports of histologic findings for ASACA specimens obtained from dogs. Rectal examination should be included in routine physical examinations of dogs and may be useful as a simple screening test to detect early-stage ASACA, especially in older dogs.

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## Footnotes

a. Target Retrieval Solution S1699, Dako North America Inc, Carpinteria, Calif.
b. Ki-67 antigen (clone MB-1), Dako North America Inc, Carpinteria, Calif.
c. Purified mouse anti–E-cadherin (clone 36), BD Biosciences, San Jose, Calif.
d. 4+ Detection System, Biocare Medical, Concord, Calif.
e. NovaRED for peroxidase (SK-4800), Vector Laboratories, Burlingame, Calif.
f. Prism, version 6.0c, GraphPad Software Inc, La Jolla, Calif.

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**From this month's *AJVR***

### Evaluation of self-injurious behavior, thermal sensitivity, food intake, fecal output, and pica after injection of three buprenorphine formulations in rats (*Rattus norvegicus*)

Molly Allen and Rebecca A. Johnson

**OBJECTIVE**
To assess effects of buprenorphine hydrochloride (BH), sustained-release buprenorphine (SRB), and high-concentration buprenorphine (HCB) formulations in healthy rats.

**ANIMALS**
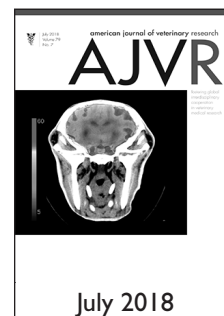8 Sprague-Dawley rats.

**PROCEDURES**
In a crossover-design study, rats received BH (0.05 mg/kg), SRB (1.2 mg/kg), HCB (0.30 mg/kg), or 5% dextrose solution (0.2 mL/kg), SC, once. Self-injurious behavior and thermal sensitivity (hind limb withdrawal latencies) were assessed prior to injection (time 0) and 1, 4, 8, 12, and 24 hours after injection. Food intake, kaolin intake, and fecal output were measured over 12-hour light and dark periods before and after each treatment. Values were compared among treatments and time points.

**RESULTS**
Self-injurious behavior was detected with all buprenorphine treatments; scores were greater at all time points for 12 hours after HCB and 24 hours after SRB administration than at time 0. Percentage change in hind limb withdrawal latencies from time 0 was higher with BH and HCB 1 hour after injection than at other time points. Postinjection light-period food intake was higher (BH and HCB) and dark-period food intake was lower (BH, HCB, and SRB), compared with preinjection values for the same treatments. For SRB, postinjection light-period kaolin intake was greater than the preinjection value, and postinjection light- and dark-period kaolin intake was greater than that for other treatments.

**CONCLUSIONS AND CLINICAL RELEVANCE**
Hypoalgesic effects were briefly observed after administration of BH or HCB in healthy rats; adverse effects were detected in some rats with all buprenorphine formulations. Studies comparing effects of BH, SRB, and HCB in rats undergoing surgery or other noxious stimuli are indicated to determine clinical benefits in this species. (*Am J Vet Res* 2018;79:697–703)

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