Canine Hemangiosarcoma

Amy R. Kraft
Class of 2018
Clinicopathologic Conference
February 16, 2017

CPC Advisors

Hayley Gallaher, DVM
Resident, Small Animal Surgery

Katie Cooley-Lock, DVM, MS
Resident, Small Animal Internal Medicine

COLLEGE *of*
VETERINARY MEDICINE
MISSISSIPPI STATE UNIVERSITY™

Introduction

Hemangiosarcoma (HSA) is a highly malignant tumor derived from vascular endothelial cells and is characterized by early and aggressive metastasis. HSA is considered a common tumor type in dogs, comprising up to 7% of noncutaneous primary malignant neoplasms.[14] The neoplasm is usually seen in middle-aged to older dogs, with a mean age of occurrence at 8 to 13 years. Large breeds in general are considered to be at a higher risk than small breeds, and while numerous breeds are over-represented, the Golden Retriever and German Shepherd Dog are considered to be at the highest risk. Golden Retrievers, in fact, have a 1 in 5 lifetime risk of developing HSA, while Irish Setters (the breed of interest in this case report) are estimated to have a 1 in 34 risk of developing HSA.[3, 19] Due to a strong breed predisposition, a genetic etiology for HSA has been suggested, and mutations in p53 and the phosphatase and tensin homolog gene (or PTEN), two important tumor suppressor genes, have recently been demonstrated in canine HSA.[1, 9]

History

Seamus, a 7-year-old male intact Irish Setter, was presented to the MSU-CVM Internal Medicine Department on April 28, 2017 after referral for a distended abdomen with free fluid noted. Seamus had repeated episodes of lethargy prior to referral, and bloodwork, urinalysis, total T4, symmetric dimethylarginine (SDMA) values, and tick PCR panel values performed at his primary veterinarian were all within normal limits. A small, raised bump was also noted on his dorsum (documented at multiple visits), and a fine needle aspirate (FNA) of the mass was consistent with subcutaneous HSA. Histopathology was recommended for definitive diagnosis, and a mass removal was scheduled for April 28. However, on presentation for surgery, Seamus was extremely lethargic and depressed. An abdominal focused assessment with sonography for

trauma (aFAST) scan of the abdomen revealed free peritoneal fluid, and Seamus was referred to MSU-CVM for further workup.

Presentation

Upon presentation, Seamus was quiet, but alert and responsive. He had an ideal body condition score of 5/9. For a dog of his size, he was considered moderately tachycardic at 132 beats per minute. His respiratory rate (32 breaths per minute) and temperature (100.7°F) were within normal limits. His mucous membranes were slightly pale and tacky with a capillary refill time of less than 2 seconds. His heart and lungs auscultated normally. A well-circumscribed, freely movable, soft mass was present on the dorsum lateral to the spine and measured approximately 2 x 2 x 1.5 cm. Rectal examination revealed an enlarged but symmetrical prostate. The abdomen was tense on palpation. FAST scan of the abdomen revealed a fluid score of 3/4, while FAST scan of the thorax showed no evidence of pericardial or pleural effusion.

An abdominocentesis was performed to characterize the effusion, and bloody, non-clotting fluid was removed. A packed cell volume (PCV) of the abdominal fluid was found to be 44%, while the PCV of the peripheral blood was 40%. A serum lactate was normal at 0.9 mmol/L. Complete blood count (CBC) values showed a moderate lymphopenia at 468/uL (reference: 1200-6500) and mild thrombocytopenia at 134 K/ul (reference: 160-650), and chemistry values were unremarkable. A coagulation profile was also submitted and found to be within normal limits.

Seamus's history and presentation were consistent with hemoabdomen associated with a ruptured abdominal mass, with visceral HSA being considered most likely. Clinical signs can be subtle and are usually related to episodic weakness or acute collapse associated with hemorrhage from the mass, followed by recovery when the blood is reabsorbed from the abdomen.[14] Other

signs consistent with a ruptured HSA include tachycardia, tachypnea, mucous membrane pallor, abdominal distention or discomfort, anorexia, and weight loss.[14]

The heart is considered the second most common location for HSA after the spleen, with most located in the right atrium or auricle.[10] Additionally, it has been reported that approximately 25% of dogs with HSA in the spleen also have another primary HSA within the heart.[1, 14] Unsurprisingly, cardiac HSA may present with different clinical signs than those associated with visceral tumors, which manifest as a result of pericardial effusion and subsequent cardiac tamponade, and possible signs of right-sided heart failure. These include abdominal distension, jugular pulses, muffled heart sounds, and dyspnea.[14]

Other possible clinical signs vary based on the specific location of the tumor. For example, subcutaneous HSA rarely results in overt clinical signs, although these tumors are prone to hemorrhage if traumatized. Dogs with HSA are also prone to disseminated intravascular coagulation (DIC), and petechiae formation may result in these animals. Occasionally an animal may die acutely with no clinical signs if a tumor ruptures acutely.[14]

Differential Diagnoses

Due to the presence of blood within the abdominal cavity, differentials for hemoabdomen were considered. Hemoabdomen cases can generally be classified as traumatic or nontraumatic. Nontraumatic hemoabdomen has been associated with benign or malignant intra-abdominal neoplasia, coagulopathies, gastric dilatation and volvulus, liver lobe torsion, splenic torsion, and iatrogenic (post-surgical) etiologies.[1] Seamus's age, physical exam, history, and initial diagnostics made visceral HSA the top differential. While the possibility of a benign lesion such as a splenic hemangioma or hematoma still existed, studies suggest that nontraumatic hemoabdomen is considerably more often caused by malignant neoplasia, with 70% of

hemoabdomen cases in one study resulting from HSA.[12] However, a diagnosis of HSA cannot be made solely on detection of a hemoabdomen, and further diagnostics should be pursued to determine the source of bleeding and provide an accurate prognosis and possible treatment options to the owner.[20]

Pathophysiology

The etiology and biological behavior of HSA is somewhat variable based on the specific organ affected. Cutaneous HSA, for example, is associated with ultraviolet light exposure in poorly pigmented dogs, such as Whippets and Pitbulls.[17] Subcutaneous and visceral HSAs have not been associated with ultraviolet light exposure and carry a significantly worse prognosis.[14]

As this neoplasm can arise in any tissue with blood vessels, primary sites of occurrence are widespread, with the most common sites being the spleen (50-65%), right atrium (3-25%), subcutaneous tissue (13-17%), and liver (5-6%).[14] Metastatic dissemination and local infiltration occur early in disease, either hematogenously or via local seeding following tumor rupture, and more than 80% of canine patients demonstrate overt metastasis at clinical presentation.[1, 14] While the liver, omentum, and lungs are the most common sites of metastasis, other reported sites include the bone, eye, and, as is likely in this case report, the adrenal glands.[14] HSA is also the most common metastatic sarcoma in the canine brain, with 14% of patients having brain metastasis in one study, and these dogs may therefore present with neurological signs.[18, 23]

Diagnostic Approach & Considerations

Although a fine-needle aspirate of a cutaneous or subcutaneous tumor may be all that is required for a probable diagnosis of HSA, it is often non-diagnostic due to blood contamination and the poorly exfoliative nature of sarcomas. The risk of splenic rupture or tumor seeding must also be considered when a decision is made to aspirate a splenic mass.[14] While prominent

hemorrhage and the presence of abnormal mesenchymal cells may increase clinical suspicion, definitive diagnosis of HSA requires further diagnostics. Common tests for diagnosis and staging include a CBC, chemistry panel, urinalysis, coagulation profile, three-view thoracic radiographs, abdominal radiographs and ultrasound, echocardiogram, and electrocardiography.

Abnormalities that may be seen on a CBC in a dog with HSA include evidence of a regenerative anemia (i.e. anisocytosis, polychromasia, reticulocytosis), acanthocytosis (seen in up to 50% of dogs with HSA), and schistocytes due to microangiopathic changes.[14] However, the anemia may be characterized as non-regenerative if bleeding occurred acutely and the bone marrow has not had time to respond. Thrombocytopenia is also a common finding in 97% of dogs with HSA, which may result from immune-mediated mechanisms, severe hemorrhage, or DIC. [4, 14]

Serum chemistry abnormalities are usually not specific to HSA, but may be useful in determining organ involvement, such as increases in serum ALP and ALT reflecting possible infiltration of the liver. Hypoglycemia may also result as a paraneoplastic syndrome. Other findings may include panhypoproteinemia from blood loss, and pre-renal azotemia. Clotting factors may also be prolonged, and may indicate impending DIC.[5]

Radiographs of the abdomen and thorax may exhibit pleural or peritoneal effusion secondary to hemorrhage of the primary tumor or heart failure associated with cardiac tamponade. A globoid cardiac silhouette will likely be present in cases of pericardial effusion. Thoracic radiography is also useful in evaluating patients for pulmonary metastatic disease, with both nodular and diffuse miliary patterns being consistent with metastatic HSA.[14]

Abdominal radiographs may demonstrate intra-abdominal masses or other evidence such as splenomegaly or hepatomegaly. Generalized loss of detail is consistent with peritoneal

effusion with hemoabdomen being the most common reason for dogs with visceral HSA to present to a veterinarian. Abdominal ultrasonography is also useful for the identification of primary masses or evidence of metastasis, although HSA cannot be differentiated from benign lesions such as hemangiomas or hematomas. However, clinical suspicion is higher in the presence of hemoabdomen, anemia, and abnormal red blood cell morphology. The commonly cited "two-thirds" rule summarizes the odds of HSA with a splenic mass: approximately two-thirds of dogs with splenic masses have a malignant tumor, and of these dogs, two-thirds will have HSA. The remaining patients typically have benign disease and thus have a dramatically improved prognosis with splenectomy alone. [14]

In select cases, additional imaging may be required for identification of suspected neoplasia based on physical exam findings. For example, limb radiographs may be pursued in cases of lameness suggesting a bone lesion, and magnetic resonance imaging (MRI) may be elected if neurological signs are present suggesting brain metastasis. MRI or computed tomography (CT) may also be pursued for surgical planning for removal of subcutaneous or intramuscular HSA.[14]

Given the high probability of cardiac involvement, echocardiography and ECG are useful tests for cardiac evaluation.[14] Echocardiography is sensitive for detection of cardiac masses, and ECG can detect abnormalities such as ventricular arrhythmias, which are more often associated with splenic disease than cardiac tumors; however, arrhythmias are also possible due to local tumor involvement of the cardiac conduction system, and pericardial effusion can result in electrical alternans.[14] In cases of pericardial effusion, a pericardiocentesis may be performed to correct arrhythmias as well as to collect a fluid sample for diagnostics. It should be noted that

hemorrhagic samples are consistent with both HSA and idiopathic pericardial effusion, and it is difficult to differentiate neoplastic and non-neoplastic etiologies based on cytology alone.

The definitive diagnosis of HSA can only be confirmed with histopathology. Excisional biopsies are preferred, and can therefore be considered both a diagnostic and therapeutic procedure. A full abdominal exploratory should also be performed when surgically removing visceral tumors, and biopsies of any suspicious lesions within the spleen, liver, or omentum should be performed at this time. Biopsies of normal-appearing liver and spleen should also be considered due to the high rate of metastasis to these highly vascular organs.[14]

Treatment & Management

Surgical removal of the mass, if amenable to surgery, is often the recommended initial treatment in most cases of HSA, as an actively bleeding tumor is the most common cause of presentation.[26] While almost all primary locations carry a poor long-term prognosis, surgery may still be performed for immediate palliation of clinical signs, and a pericardectomy may be considered for cardiac HSA with associated pericardial effusion.[14] The exception to this rule is cutaneous HSA, which tends not to metastasize and carries a better prognosis with a median survival time (MST) of 780 days with surgery alone. Dogs typically recover well with surgery and supportive care (i.e. fluids, anti-arrhythmics, blood products) and may be non-clinical for months following surgery. Most dogs will ultimately die of metastatic disease to the lungs or viscera that rupture and results in further hemorrhage. MST for dogs with HSA varies based on location of the tumor, but typically is from weeks to months regardless of treatment modality. Splenic HSA treated with surgery alone carries the shortest survival time and ranges from 19 to 86 days.[14]

Chemotherapy may be used to treat metastatic disease following surgical removal of the primary tumor. Doxorubicin-based chemotherapy is considered to have the greatest chemotherapeutic efficacy against canine HSA as either a single agent or in combination with other agents. It is important to note, especially in financially restrictive cases, that survival times seem to be similar when comparing doxorubicin alone to combination protocols with vincristine and cyclophosphamide.[4, 16, 26] As with all chemotherapeutic protocols, balancing the risk of side effects and benefits of therapy should be considered, with common toxicities including neutropenia and gastrointestinal signs.[4, 16] Doxorubicin can cause severe perivascular sloughing if extravasation occurs, and is also known to cause dose-dependent cardiotoxicity.[4] As such, echocardiography is a common diagnostic tool prior to administration to reveal any underlying systolic dysfunction that would contraindicate doxorubicin.[15] One study indicates that epirubicin may be just as efficacious as doxorubicin-based protocols and can be considered as an alterative in dogs with preexisting cardiac disease, although this protocol may result in a higher degree of gastrointestinal effects.[6] While chemotherapy is effective in extending survival times in most cases, expected increases are usually modest (weeks to months), and one-year survival times are not improved. Efficacy of chemotherapy is also dependent on the stage of cancer, with stage III HSA having a considerably shorter MST than stage I (87 vs. 250 days).[16] The location of the tumor can also affect the efficacy of chemotherapeutic treatment, as MSTs for dogs with cardiac HSA remain notably lower than those achieved for visceral presentations, likely due to the difficulty in obtaining local control for a cardiac tumors compared to a splenic or hepatic mass.[10]

Immunomodulators may also be considered in the treatment of HSA, especially when used in combination with chemotherapy. A mixed bacterial vaccine and liposome-encapsulated muramyl tripeptide (L-MTP) have both been used to treat HSA and have reportedly increased

MST when used with surgery and chemotherapy. Although increases in MST were modest, results suggest that immunotherapy may be an area of promising research for adjuvant treatment of HSA.[21, 22]

Due to the lack of effective adjuvant therapies for HSA, novel treatment options such as *Yunnan Baiyao* have been explored in recent years. *Yunnan Baiyao*, a well-known Chinese herbal medicine, has been utilized for its anti-inflammatory, hemostatic, and pain relieving properties in people for over 100 years, and has been shown to improve clotting and enhance platelet function. As such, it has been anecdotally reported to prolong MST and control bleeding in dogs with HSA. One *in-vitro* study found that *Yunnan Baiyao* causes dose and time dependent HSA cell death.[24] However, while evidence supports its safety, there is little evidence that would suggest *Yunnan Baiyao* improves MST or clinical signs in dogs with HSA.[11]

Case Outcome

Following diagnosis of a hemoabdomen, Seamus was started on IV fluid therapy of Plasmalyte and a 500 mL bolus was administered before starting a fluid rate twice that of maintenance. Doses of methadone and maropitant citrate were also administered. Thoracic radiographs were taken, and revealed three sharply marginated, soft-tissue opacity nodules consistent with metastatic neoplasia. Abdominal radiographs indicated the presence of abdominal effusion, and also revealed mild prostatomegaly consistent with physical exam findings and bilateral coxofemoral joint osteoarthritis. A follow-up abdominal ultrasound confirmed free fluid. The ventral margin of the spleen was indistinct, and the left adrenal gland could not be visualized. Although the source of the bleeding could not be identified on ultrasound, a ruptured splenic mass was considered most likely. A focused echocardiogram was

also performed, and no mass in the right atrium or evidence of pericardial effusion were identified.

Despite the likelihood of pulmonary metastasis and high clinical suspicion of HSA, the owner elected to pursue surgical removal of the suspected splenic mass. Under general anesthesia, Seamus was placed in dorsal recumbency and an exploratory laparotomy was performed. Approximately 750 mL of hemorrhagic fluid was removed from the abdomen, and a large hematoma was located at the head of the spleen. The hematoma was removed and no evidence of bleeding could be seen from the spleen, which was grossly normal in appearance. Upon further exploration, an approximately 4 cm x 4 cm mass was noted on the left adrenal, which was ulcerated and freely hemorrhaging. No other abnormalities were noted. The adrenal mass was then dissected from the surrounding tissue and vasculature. Moderate blood loss occurred, and a blood transfusion was started during surgery. A small biopsy was also taken from the liver with a guillotine method, and both the excised adrenal gland and liver biopsy were submitted for histopathology.

Seamus recovered from anesthesia uneventfully and completed his blood transfusion with no complications. An iStat was taken post-operatively, which was unremarkable. A PCV/total protein (TP) were taken as well, which was normal at 34/5.9. IV fluid therapy was continued at maintenance rates. Non-invasive blood pressures were monitored periodically overnight and found to be normal, and pain was controlled with hydromorphone. Dexamethasone sodium phosphate was also administered to provide temporary compensation for removal of an adrenal gland.

Although quiet, Seamus's physical exam was within normal limits the morning following surgery (April 29). A chemistry panel was submitted to recheck values and showed a mild

hypoproteinemia at 4.8 g/dl (reference: 5.5-8.0) and a mild hypoalbuminemia at 2.3 g/dl (reference: 2.5-3.9). A PCV/TP was also rechecked and found to have increased to 36/6.0. Seamus continued to recover well in hospital and was discharged on May 1, 2017 with Tylenol 4, a tapering dose of prednisolone, and instructions to return to have his incision rechecked.

Histopathology results became available before Seamus's recheck. A mesenchymal neoplasm forming irregular blood-filled structures markedly expanded the adrenal gland, thus confirming a diagnosis of HSA. Given the previously noted mass on Seamus's dorsum as well as the high rate of adrenal metastasis (21% in one study), it is suspected that this was the primary tumor site, and metastasis to the lungs and adrenal glands occurred secondarily.[7] While the liver biopsy showed mild hepatitis, no evidence of metastasis was found in the provided sample.

Seamus returned on May 9, 2017 for his incision recheck, as well as removal of the previously noted mass on his dorsum and a newly discovered mass on his left lateral thigh. The abdominal incision had healed nicely, and Seamus was doing well following surgery. A CBC and chemistry panel were submitted and found to be unremarkable. He was once more placed under general anesthesia and both masses were excised with wide margins and submitted for histopathology. There were no complications and Seamus was discharged the following morning. Another recheck appointment was made, as well as an oncology consultation with the MSU-CVM Internal Medicine service. The mass from the thigh was found to be a benign infundibular cyst, while the mass from the dorsum was confirmed to be a subcutaneous HSA.

Seamus returned on May 24, 2017. His incisions were found to have healed nicely, although a seroma had formed at the site on his back. Blood was again drawn for a CBC and chemistry panel, which were unremarkable. A fractional shortening and ECG were also performed and were within normal limits. Three view chest radiographs were repeated and

revealed increased numbers of sharply marginated, soft tissue opaque nodules, indicating progression of metastasis in the lungs. On abdominal ultrasound, further evidence of metastasis was evident. Given the progression of metastasis, a poor prognosis was provided, and while multiple treatment options were discussed with the owner, including a single agent protocol of doxorubicin, metronomic chemotherapy, and palliative care, no decision was reached.

Unfortunately, despite surgical removal of the adrenal tumor, Seamus was euthanized on May 26, 2017 following another internal bleed less than one month following surgery. The outcome of Seamus's case is consistent with the expected prognosis of metastatic HSA, with one-year survival for dogs with HSA being less than 10%. Higher stage disease, such as evidence of metastasis, as well as hemoabdomen associated with a bleeding tumor are negative prognostic factors, both of which were present in Seamus's case. As evidenced by this case, canine HSA is an incredibly aggressive neoplasia, and prognosis is invariably poor for long-term survival in almost all cases.

References

1. Clifford CA and de Lorimier LP. Canine hemangiosarcoma. In: *Kirk's Current Veterinary Therapy*. St. Louis, Elsevier, 2014: 392 – 397.
2. Clifford CA, Mackin AJ, Henry CJ. Treatment of canine hemangiosarcoma: 2000 and beyond. J Vet Intern Med 2000; 14: 479–485.
3. Glickman LT, Glickman N, Raghavan M (2003) The Irish Setter Club of America 2003 National Health Survey. Irish Setter Club of America, Inc.
4. Hammer AS, Couto CG. Filppi J, et al. Efficacy and toxicity of VAC chemotherapy (vincristine, doxorubicin, and cyclophos- phamide) in dogs with hemangiosarcoma. J Vet Intern Med 199I; 5:160-166.
5. Hammer AS, Couto CG, Swardson C, et al. Hemostatic abnormalities in dogs with hemangiosarcoma. J Vet Intern Med 1991;5:11–4.
6. Kim SE, Liptak JM, Gall TT, Monteith GJ and Woods JP. Epirubicin in the adjuvant treatment of splenic hemangiosarcoma in dogs: 59 cases (1997-2004). *Journal of the American Veterinary Medical Association* 2007; 231: 1550 – 1557.
7. Labelle P, De Cock HEV. Metastatic tumors to the adrenal glands in domestic animals. *Vet Pathology* 2005; 42: 52-58.
8. Lana S, U'ren L, Plaza S, Elmslie R, Gustafson D, Morley P, *et al.* Continuous low-dose oral chemotherapy for adjuvant therapy of splenic hemangiosarcoma in dogs. *Journal of Veterinary Internal Medicine* 2007; 21: 764–769.
9. Mayr B, Zwetkoff S, Schaffner G, and Reifinger M, "Tumour suppressor gene p53 mutation in a case of hemangiosarcoma of a dog," *Acta Veterinaria Hungarica*, vol. 50, no. 2, pp. 157-160, 2002.

10. Mullin CM, Arkans MA, Sammarco CD, Vail DM, *et al*. Doxorubicin chemotherapy for presumptive cardiac hemangiosarcoma in dogs. *Veterinary and Comparative Oncology* 2016; 14: 171-183.
11. Murphy LA, Panek CM, Bianco D, Nakamura RK. Use of Yunnan Baiyao and epsilon aminocaproic acid in dogs with right atrial masses and pericardial effusion. *Journal of Veterinary Emergency and Critical Care* 2017; 27: 121-126.
12. Pintar J, Breitschwerdt EB, Hardie EM and Spaulding KA. Acute nontraumatic hemoabdomen in the dog: a retrospective analysis of 39 cases (1987–2001). *Journal of the American Animal Hospital Association* 2003; **39**: 518–522.
13. Prymak C, McKee LJ, Goldschmidt MH, et al. Epidemiologic, clinical, pathologic, and prognostic characteristics of splenic hemangiosarcoma and splenic hematoma in dogs: 2I7 cases (1985*). J Am Vet Med Assoc* 1988; 193:706-712.
14. Smith AN. Hemangiosarcoma in dogs and cats. *Veterinary Clinics of North America: Small Animal Practice* 2003; 33: 533–552.
15. Sorenmo KA, Jeglum KA and Helfand SC. Chemotherapy of canine hemangiosarcoma with doxorubicin and cyclophosphamide. *Journal of Veterinary Internal Medicine* 1993; 7: 370–376.
16. Sorenmo KU, Baez JL, Clifford CA, Mauldin E, Overley B, Skorupski K, *et al.* Efficacy of a dose-intensified doxorubicin protocol in canine hemangiosarcoma. *Journal of Veterinary Internal Medicine* 2004; 18: 209 – 213.
17. Szivek A, Burns RE, Gericota B, et al. Clinical outcome in 94 cases of dermal haemangiosarcoma in dogs treated with surgical excision: 1993–2007. *Veterinary and Comparative Oncology* 2011; 10: 65-73.

18. Synder JM, Lipitz L, Skorupski KA, Shofer FS, Van Winkle TJ. Secondary intracranial neoplasia in the dog: 177 cases (1986-2003). *J Vet Intern Med* 2008; 22:172–177

19. Tamburini BA, Trapp S, Phang TL, Schappa JT, Hunter LE, Modiano JF. Gene expression profiles of sporadic canine hemangiosarcoma are uniquely associated with breed. *PLoS One*. 2009; 4: e5549.

20. Thompson, Mark S. "Abdominal Effusion and Ascites." *Small Animal Medical Differential Diagnosis*. Saunders Elsevier, 2007, pp. 3-4.

21. U'Ren LW, Biller BJ, Elmslie RE, Thamm D, Dow SW. Evaluation of a novel tumor vaccine in dogs with hemangiosarcoma. *J Vet Intern Med* 2007; 21:113–120.

22. Vail DM, MacEwen EG, Kurzman ID, et al. Liposome- encapsulated muramyl tripeptide phosphatidylethanolamine adjuvant immunotherapy for splenic hemangiosarcoma in the dog: a randomized multi-institutional clinical trial. Clin Cancer Res 1995; 1: 1165–1170.

23. Waters DJ, Hayden DW, Walter PA. Intracranial lesions in dogs with hemangiosarcoma. *J Vet Intern Med* 1989; 3: 222–30.

24. Wirth KA, Kow K, Salute ME, Bacon NJ, Milner RJ. In vitro effects of Yunnan Baiyao on canine hemangiosarcoma cell lines. *Veterinary and Comparative Oncology* 2016; 14: 281-294.

25. Cleveland MJ, Casale S. Incidence of malignancy and outcomes for dogs undergoing splenectomy for incidentally detected nonruptured splenic nodules or masses: 105 cases (2009-2013). *J Am Vet Med Assoc* 2016; 284: 1267– 1273.

26. Ogilvie GK, Powers BE, Mallinckrodt CH, Withrow SJ. Surgery and doxorubicin in dogs with hemangiosarcoma. *J Vet Intern Med* 1996;10:379–84.