NOTE   *Surgery*

Secondary Erythrocytosis Associated with Schwannoma in a Dog

Akiko YAMAUCHI[1], Tamiko OHTA[1], Taro OKADA[2], Manabu MOCHIZUKI[1], Ryohei NISHIMURA[1], Satoru MATSUNAGA[1], Hiroyuki NAKAYAMA[2], Kunio DOI[2] and Nobuo SASAKI[1]

[1]*Laboratories of Veterinary Surgery and* [2]*Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan*

ABSTRACT. An 11-year-old, spayed female mixed-breed dog showed clinical signs of right forelimb lameness and pain by palpation around the neck. Radiography and computed tomography (CT) revealed an extradural mass at the 6th and 7th cervical vertebrae, which compressed the spinal cord. The mass was surgically removed and histopathologically diagnosed as schwannoma. The dog recovered her normal gait after hemilaminectomy and removal of the mass. Ten months after the surgery, the tumor recurred with absolute erythrocytosis and was surgically removed again. This removal temporarily resolved the erythrocytosis with a decrease in plasma erythropoietin (EPO) concentration. EPO protein was detected immunohistochemically in the tumor cells. Erythrocytosis in this dog may be caused by ectopic EPO produced in the schwannoma tissues.
KEY WORDS: canine, erythrocytosis, schwannoma.

Schwannoma is a common tumor of nerve roots and peripheral nerves in dogs that arises from Schwann cells, which are the component of the cellular sheath surrounding peripheral nerve axons. In dogs, schwannoma is most likely to develop at the brachial plexus or its nerve root areas. The tumor generally grows slowly, expanding along the nerve and its branches, and metastasis is quite uncommon [10]. Surgical excision has been successful in a few isolated cases [1], but local recurrence is common if complete resection can not be achieved [19]. The median survival time after diagnosis in dogs with this tumor at the brachial plexus or nerve roots is reported to be 12 or 5 months, respectively [2].

Polycythemia (erythrocytosis) is defined as a relative or absolute increase in peripheral erythrocytes. Relative erythrocytosis is caused by dehydration, while absolute polycythemia is caused by an increased total red blood cell (RBC) mass. The latter is divided into primary and secondary polycythemia [9, 14]. Primary polycythemia (polycythemia vera) is considered a myeloproliferative disease and, in human, is often accompanied by increased white blood cell (WBC) and platelet counts and decreased plasma erythropoietin (EPO) concentration [8]. In dogs with primary polycythemia vera, EPO concentrations are low or within the normal range [9]. Secondary polycythemia is further subdivided into appropriate and inappropriate categories. Secondary appropriate polycythemia is a consequence of persistent systemic hypoxia commonly caused by pulmonary and cardiac disorders or venoarterial shunts. Secondary inappropriate polycythemia refers to renal diseases or neoplasia without systemic hypoxia [9, 14]. A variety of neoplasms, including renal cell carcinoma [13], hepatocel-

lular carcinoma [11], Wilms' tumors [3], cerebellar hemangioblastomas [17], and uterine leiomyomas [18] have been associated with erythrocytosis in humans. In dogs, there have been several reports on secondary erythrocytosis associated with renal neoplasm, including carcinoma [15], lymphosarcoma [12], fibrosarcoma [7], and adenocarcinoma [5], as well as with nasal fibrosarcoma [4] and cecal leiomyosarcoma [16].

An 11-year-old, spayed female mixed-breed dog was referred to the Veterinary Medical Center at the University of Tokyo with the clinical signs of right forelimb lameness and neck pain unresponsive to glucocorticoid therapy for the past 2 months. The dog was ambulatory and a mild deficit of postural reaction of the forelimbs was noted. Blood chemistry profile and serial hemograms demonstrated increased alkaline phosphatase activity (1,641 U/l), presumably caused by prolonged administration of glucocorticoid, and slight increases in RBC count ($832 \times 10^4/\mu l$; reference range, 540 to $780 \times 10^4/\mu l$), packed cell volume (PCV) (51%; reference range, 37 to 54%), and hemoglobin concentration (18.4 g/dl; reference range, 13 to 19 g/dl). Cervical and thoracic survey radiographs were unremarkable. Myelography and computed tomography (CT) revealed an extradural mass at the level of 6th and 7th cervical vertebrae, which compressed the spinal cord from the right side and extended to the outside of the vertebral canal with partial destruction of the spinal bone (Fig. 1).

Surgery was undertaken to remove the mass through hemilaminectomy. At the surgery, the 6th to 7th (C6, C7) cervical vertebrae were fragile and the neoplastic mass was located ventrally in the vertebral canal, thereby compressing the spinal cord. Three weeks after surgery, the dog recovered a normal gait and the pain disappeared. The mass was histopathologically diagnosed as schwannoma.

Ten months after surgery, the dog showed quadriplegia with pain around the neck. Physical examination revealed

* CORRESPONDENCE TO: SASAKI, N., Laboratory of Veterinary Surgery, Graduate School of Agricultural and Life sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan.
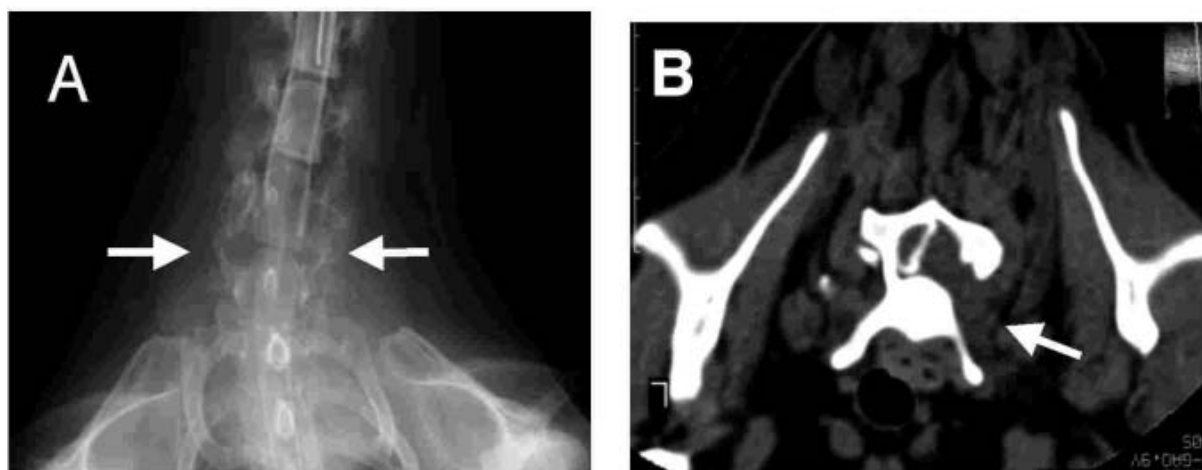
Fig. 1.   (A and B) Ventrodorsal cervical myelography (A) and computed tomography (B) at the first admission. There was an extradural mass at the 6th and 7th cervical vertebrae, which compressed the spinal cord from the right side and extended to the outside of the vertebral canal, destroying the spinal bone (arrows).
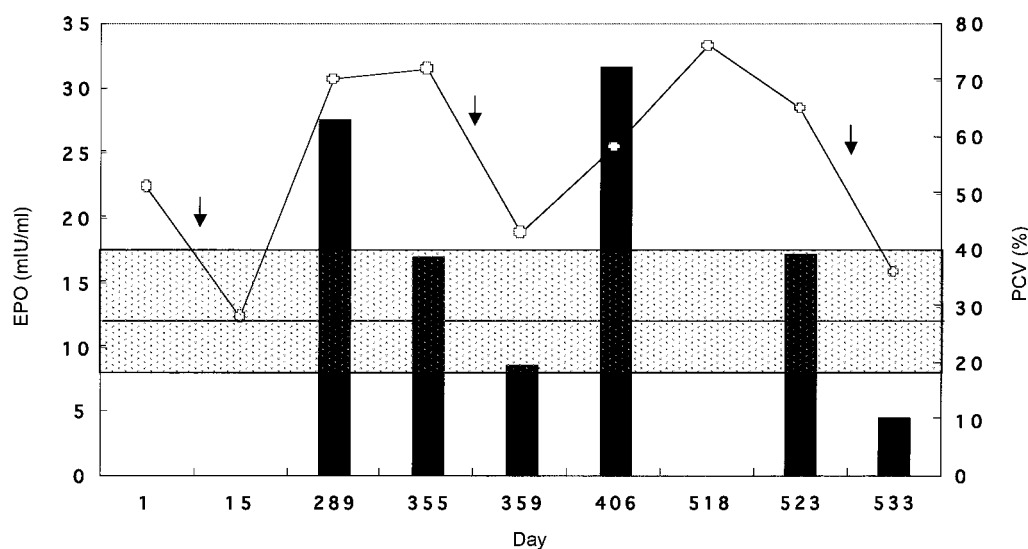

Fig. 2.   Changes in packed cell volume (PCV, open circles) and plasma erythropoietin (EPO) concentration (solid bars) in this case. Surgery was performed on days 6, 356, and 524 (arrows). The shadow indicates the reference range of plasma EPO concentration calculated from those of 6 normal beagle dogs.

congested mucous membranes and dehydration. Hematologic abnormalities included remarkable increases in RBC count ($1,315 \times 10^4/\mu l$), PCV (74%), and hemoglobin concentration (27.8 g/dl). Total plasma protein concentration (6.8 g/dl; reference range, 6.0 to 7.8 g/dl) was within the normal range. Clinical signs related to polycythemia like hemorrhage and neurological disorders were not recognized.

Cervical radiographs revealed bone destruction in the C6 and C7 vertebrae. Thoracic radiographs did not show any pulmonary or cardiac abnormality. Abdominal radiographs and ultrasonography revealed mild hepatomegaly, mild splenomegaly, and slight atrophy of the right kidney. The patient's plasma EPO concentration, determined by a commercial enzyme-linked immunosorbent assay (ELISA) kit for human (Toyoboseki Co., Osaka, Japan), was higher (27.6 mIU/mL) than those of 6 clinically normal beagle dogs (8.0 to 18.2 mIU/mL; mean 12.0 mIU/mL) (Fig. 2), suggesting that the erythrocytosis of the patient was not primary.

The dog was treated with intravenous injections of dexamethasone (1 mg/kg on the first day) and lactated Ringer's solution. The dose of dexamethasone was gradually decreased. One week later, the dog was ambulatory with upper motor neuron signs in four limbs, but RBC count ($1,107 \times 10^4/\mu l$), PCV (70%) and hemoglobin concentration
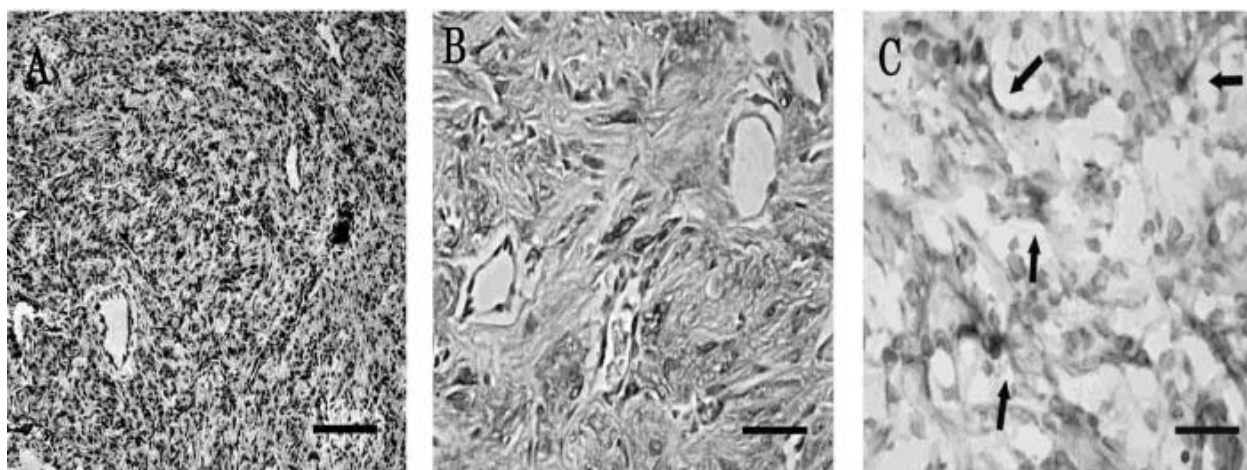
Fig. 3. Histology of schwannoma tissue surgically removed. (A) Light microscopy. A solid proliferation of spindle-shaped neoplastic cells with many vacuoles was noted. The nuclei of the neoplastic cells were hypochromatic and mildly varied in size. Haematoxylin and eosin staining. × 100, Bar=200 μm. (B) Immunohistochemistry. The cytoplasm of tumor cells was positive for S-100 protein. × 400, Bar=110 μm. (C) Immunohistochemistry. In some tumor cells, positive signals for EPO (arrows) were observed in the cytoplasm. × 400, Bar=110 μm.

(24.3 g/dl) were still high in spite of the normal total plasma protein concentration (6.0 g/dl).

The dog was discharged and medicated with reduced dosages of prednisolone and cephalexin. However, a month later, quadriplegia and pain recurred. CT revealed a recurrence of the mass at C6 and C7, compressing the spinal cord and destroying the spine. In addition, the mass had extended ventrally between each vertebra and the trachea. A complete blood count revealed an increased RBC count ($1,157 \times 10^4/\mu l$), PCV (72%), and hemoglobin concentration (25.2 g/dl). Analysis of a blood sample from the femoral artery revealed high oxygen tension (PaO$_2$; 151 mmHg; reference range, 91 to 97 mmHg), normal arterial pH (7.38; reference range, 7.3 to 7.43), and normal carbon dioxide tension (PaCO$_2$; 38.1 mmHg; reference range, 30 to 49 mmHg). This blood sampling was performed under light anesthesia with oxygen inhalation because of the aggressive behavior of this patient, which may result in higher oxygen tension. However these data may indicate no chronic hypoxic status in this patient. The reticulocyte count was high (2.6%; reference range, 0 to 1%), indicating active erythropoiesis. From these findings and the progression of the disease, we determined that the cause of erythrocytosis was neither low oxygenation nor renal abnormality, but ectopic EPO secretion by schwannoma. After phlebotomy (150 ml) and infusion of the equivalent volume of lactate Ringer's solution, the mass was again surgically removed.

On the second day after the surgery, the RBC count ($718 \times 104/\mu l$), PCV (43%), and hemoglobin concentrations (14.9 g/dl) decreased, as did the plasma EPO concentration (8.6 mIU/mL) (Fig. 2). After the second surgery, the dog recovered gradually, and was ambulatory without pain on the tenth day after surgery.

Five months after the second surgery, the dog showed quadriplegia again. The dog showed pain around the neck by palpation, severe muscular atrophy of the forelimbs, and congested mucous membranes. Neurological examination revealed no deep pain in the forelimbs. The RBC count ($1,324 \times 10^4/\mu l$), PCV (76%), and hemoglobin concentration (27.2 g/dl) had increased again, though plasma EPO concentration was not measured. CT revealed that the tumor mass extended ventrally around the trachea. Therefore, at the owner's request, we removed the mass using a ventral approach to decompress the nerve and relieve the pain. Ten days after the surgery, the pain was relieved but deep pain of the left forelimb did not recover and the dog could not walk. Three months after the third surgery, the dog died at home. Necropsy could not be performed.

The excised tumor tissues in the second surgery were fixed in 10% neutral buffered formalin, or were directly embedded in a fixative (O.C.T compound, Sakura, Tokyo, Japan) and immediately frozen at –80°C. Several 4-μm paraffin sections of the tumor tissue were stained with hematoxylin and eosin, or with Luxol Fast Blue (LFB). We also used immunohistochemistry to detect erythropoietin and to determine the origin of the tumor using 5-μm-thick cryosections. The avidin-biotin peroxidase complex method was applied. Rabbit sera against S-100 protein (1:200; DAKO, CA, U.S.A.), against von Willebrand factor (1:200; DAKO, Glostrup, Denmark), and against human polyclonal erythropoietin (1:50; Santa Cruz Biotechnology, CA, U.S.A.) were used as primary antibodies. Mouse serum against α-smooth muscle actin (1:50; DAKO, Glostrup) was also used as a primary antibody. Biotin-labeled goat anti-mouse (1:400; KPL, Guildford, UK) and anti-rabbit IgG (1:400; KPL) were employed as second antibodies. Immunostaining was carried out by avidin-biotin-peroxidase complex (ABC) method using commercial reagents (Vector Laboratories, Burlingame, CA).

The tumor mass consisted of solid proliferation of spindle

cells, whose nuclei were hypochromatic and slightly varied in size. Mitotic figures were rarely observed. The tumor cells possessed many vacuoles in the cell body. Immunohistochemically, the cytoplasm of tumor cells was positive for S-100 protein and α-smooth muscle actin, but negative for von Willebrand factor. Some tumor cells showed positive signals for EPO in the cytoplasm (Fig. 3). From these findings, a diagnosis of schwannoma producing EPO was made.

Several mechanisms have been postulated to explain the pathogenesis of secondary inappropriate polycythemia, including tumor-induced renal hypoxia, tumor-induced systemic hypoxia, tumor impairment of EPO catabolism, and production of EPO or other erythropoietic substances by the tumor cells [9]. Although EPO is normally produced by the kidney and fetal liver, apparently increased levels of EPO polypeptide and messenger ribonucleic acid (mRNA) have been found in neoplastic cells from renal cell carcinoma [6]. In addition, immunohistochemical staining revealed the presence of EPO in the tumor tissue of hepatocellular carcinoma, but not in normal liver tissues [11]. Erythropoietin was also detected in a cerebellar hemangioblastoma at the protein and mRNA levels [17], and ectopic EPO protein was identified in a uterine leiomyoma by immunohistochemistry [18]. In dogs, a diagnosis of ectopic EPO production was based on the resolution of the erythrocytosis following tumor resection [4,5,7], measurement of EPO activity in the tumor [4], or detection of EPO protein and mRNA expression in the tumor [16].

In this case, no increases in WBC or platelet counts were found and plasma EPO concentrations were normal to high, indicating that the erythrocytosis did not result from polycythemia vera. This dog showed no clinical signs of pulmonary or cardiac disorders, systemic or local hypoxia, or renal diseases. After explorations of the mass of schwannoma, plasma EPO concentration tended to decrease and erythrocytosis tended to resolve temporarily only to recur as the tumor grew again. These findings strongly suggest that the schwannoma was the primary cause of erythrocytosis in this dog. Furthermore, EPO protein was detected in tumor cells by immunohistochemistry. We therefore suspected that this dog's secondary erythrocytosis was attributable to ectopic production of EPO by neoplastic schwannoma cells.

There have been several reports on erythrocytosis associated with tumor in dogs. The dog described in this report showed erythrocytosis, which temporally resolved after removal of the schwannoma and EPO protein was detected in the tumor cells by immunohistochemistry. To our knowledge, this is the first report on erythrocytosis secondary to schwannoma in the dog.

REFERENCES

1. Bailey, C. S. 1990. *J. Am. Vet. Med. Assoc.* **196**: 754–756.
2. Brehm, D. M., Vite, C. H., Steinberg, H. S., Haviland, J. and Winkle, T. V. 1995. *J. Am. Anim. Hosp. Assoc.* **31**: 349–359.
3. Chen, C. W., Huang, S. P., Li, Y. C. and Huang, C. H. 2001. *J. Med. Sci.* **17**: 107–111.
4. Couto, C. G., Boudriau, R. J. and Zanjani, E. D. 1989. *J. Vet. Int. Med.* **3**: 183–185.
5. Crow, S. E., Allen, D. P., Murphy, C. J. and Culbertson, R. 1995. *J. Am. Anim. Hosp. Assoc.* **31**: 29–33.
6. Da Silva, J. L., Lacombe, C., Bruneval, P., Casadevall, N., Leporrier, M., Camilleri, J. P., Bariety, J., Tambourin, P. and Varet, B. 1990. *Blood* **75**: 577–582.
7. Gorse, M. J. 1988. *J. Am. Vet. Med. Assoc.* **192**: 793–794.
8. Hammond, D. and Winnick, S. 1974. *Ann. New York Acad. Sci.* **230**: 219–227.
9. Hasler, A. H. and Giger, U. 2000. pp. 203–206. *In*: Textbook of Veterinary Internal Medicine, 5th ed. (Ettinger, S. J. and Feldman, E. C. eds.), W.B. Saunders, Philadelphia.
10. Inzana, D. K. 2000. pp. 676–677. *In*: Textbook of Veterinary Internal Medicine, 5th ed. (Ettinger, S. J. and Feldman, E. C. eds.), W.B. Saunders, Philadelphia.
11. Matsuyama, M., Yamazaki, O. Horii, K. Higaki, I., Mikami, S., Higashino, M, Oka, H., Nakai, T. and Inoue, T. 2000. *J. Surg. Oncology* **75**: 197–202.
12. Nelson, R. W., Hager, D. and Zanjani, E. D. 1983. *J. Am. Vet. Med. Assoc.* **182**: 1396–1397.
13. Nseyo, U. O., Williams, P. D. and Murphy, G. P. 1986. *Urology* **28**: 301–306.
14. Ogilvie, G. K. Paraneoplastic syndrome. pp. 498–506. *In*: Textbook of Veterinary Internal Medicine, 5th ed. (Ettinger, S. J. and Feldman, E. C. eds.), W.B. Saunders, Philadelphia.
15. Peterson, M. E. and Zanjani, E. D. 1981. *J. Am. Vet. Med. Assoc.* **179**: 995–996.
16. Sato, K., Hikasa, Y., Morita, T., Shimada, A., Ozaki, K. and Kagota, K. 2002. *J. Am. Anim. Hosp. Assoc.* **220**: 486–490.
17. So, C-C. and Ho, L-C. 2002. *Am. J. Hematol.* **71**: 346–347.
18. Suzuki, M., Takamizawa, S., Nomaguchi, K., Suzu, S., Yamada, M., Igarashi, T. and Sato, I. 2001. *Br. J. Hematol.* **113**: 49–51.
19. Withrow, S. J. 1996. pp. 410–419. *In*: Small Animal Clinical Oncology, 2nd ed. (Withrow, S. J. and MacEwen, E. G. eds.), W.B. Saunders, Philadelphia.