

## Use of S-100 and Chromogranin A Antibodies as Immunohistochemical Markers on Detection of Malignancy in Aortic Body Tumors in Dog

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**ABSTRACT.** To define the characteristics of malignancy we performed routine histology and an immunohistochemical study on seventeen aortic body tumors in dogs. We essayed tumors using a panel of immunohistochemical markers: neuron specific enolase (NSE), chromogranin A (CrA) and S-100. Among 17 cases, the neoplastic cells were positive for NSE (17 cases, 100%), S-100 (9 cases, 53%), and CrA (8 cases, 47%), respectively. The sustentacular cells density and chief cell staining intensity were both inversely related to tumor grade. The most relevant data was consistent with a negative staining of S-100 correlated with absence or decreased number of sustentacular cells in tumors grade III. This report indicates that the immunohistochemical panel has utility for the diagnosis of chemodectoma and the negative staining to CrA and S-100 markers in tumors grade III expresses an indication of malignant behaviour of the tumor.  
**KEY WORDS:** aortic body tumor, immunohistochemistry, tumor grade.

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Aortic chemodectoma is a tumor of the aortic bodies, classified as neoplasia of the sensory receptors in the circulatory system under the name of paraganglioma. There are a few of these organs found in several sites in the body of animals, but tumors often arise from the aortic bodies, especially in dogs [14]. The function of the aortic body is connected to the chemoreceptive system (parasympathetic paraganglia): in association with the carotid body they are sensible to blood pressure and chemical composition [11]. Chemoreceptor organs in general contain two major types of cells: neuro-endocrine cells (Type I) and supporting or sustentacular cells (Type II). The cells Type I are considered as chief cells and they contain numerous secretory granules (catecholamine and serotonin). Integrated to the typical histology pattern of these paraganglia there is a variable number of connective tissue cells, vascular cells, Schwann cells, myelinated or unmyelinated nerve fibers and intrinsic neurons [31]. The chronic hypoxia seems to be involved in the pathogenesis of the tumor. High prevalence of this neoplasia is expressed in brachiocephalic dog, as matter of the upper respiratory conformation [11, 30].

Occasional aortic body tumors have been reported in human [12, 26], cat [8, 27], horse [5, 22], cow [20, 33] and duck [29]. The tumors in animals are defined as non-functional and space-occupying lesions [24]. The aortic chemodectoma in dogs are usually benign but can spread into metastasis to different sites: including the lungs, myocardium, spleen, liver and bone [4, 9, 25]. The present paper describes the histological features and immunohistochemical analysis of seventeen aortic body tumors from dogs. The aim of the work was to study S-100, chromogranin A (CrA) and neuron-specific enolase (NSE) expression in aortic body tumors in dog and establish criteria for a tumor grade in this neoplasia.

**Tumors:** Formalin-fixed paraffin wax-embedded blocks from 17 canine aortic body tumors were selected from the

archive of the Veterinary Diagnostic Laboratory, College of Veterinary Medicine at the University of Turin, Italy. Cases in the study were obtained from necropsy in a spanning period of 16 years from 1989 to 2005. Three micrometer sections from each tumor were stained with hematoxylin-eosin (HE) to investigate the histological features.

The grade for each case was derived from an assessment of mitotic rate, cellular morphology and presence of infiltrative growth in the aortic or pulmonary wall, atrial myocardium or metastasis. The tumor grade of chemodectoma was expressed into three classes: I (benign); low mitotic rate (two or less mitotic figures per high field), uniform cells, no evidence of infiltrative growth or metastasis, II (malignant); low mitotic rate (two or less mitotic figures per high field), less uniform cells, evidence of locally infiltrative growth or metastasis, and III (malignant); high mitotic rate (four or more mitotic figures per high field), evidence of locally infiltrative growth or metastasis reported [3].

**Immunohistochemical analysis:** A biotin-streptavidin horseradish peroxidase detection commercial kit (BioGenex, San Ramon, U.S.A.) was used, every section was rehydrated and immunostained with polyclonal antibody to cow S-100 protein (1:1600, Dakopatts, Denmark), and mouse monoclonal antibodies to human NSE (clone BBS/NC/VI-H14, 1:100, Dako) and human Chromogranin A (clone LK 2H10, 1:100, Menarini Italy). Sections were treated with hydrogen peroxide 6% in distilled water for eight minutes to block endogenous peroxidases, later were washed off in distilled water and incubated for 5 min (3 times) at 600 W in a microwave oven in a citrate buffer (pH 6.0). The reaction product was developed with a DAB-H<sub>2</sub>O<sub>2</sub> solution (Dako Cytomation, CA, U.S.A.) for 5 min. Finally the sections were lightly counterstained with hematoxylin. Canine central nervous tissue was used as positive control for S-100 and NSE, while islet of canine pancreas were used for CrA. Staining intensity of neoplastic cells with anti CrA,

Table 1. Results for immunohistochemical staining to CrA, S-100 and NSE

Breed	Age <sup>a)</sup>	Sex <sup>b)</sup>	Grade <sup>c)</sup>	CrA	S-100	NSE
1 Boxer	10 y	M	II	+	++	+++
2 German Shepherd	10 y	FN	II	++	++	+
3 Boxer	10 y	F	II	++	+	++
4 Bassett Hound	12 y	FN	II	+	++	+++
5 Yorkshire	8 y	MN	II	+	+	+++
6 Cross Breed	13 y	M	II	+	+	++
7 Maremma Sheepdog	10 y	FN	II	+	++	++
8 Boxer	10 y	M	II	+++	+	++
9 Cross Breed	15 y	MN	III	-	-	++
10 Cross Breed	11 y	M	III	-	-	++
11 English Bulldog <sup>d)</sup>	10 y	M	III	-	+	++
12 Cross Breed <sup>d)</sup>	12 y	M	III	-	-	++
13 Schnauzer	8 y	M	III	-	-	++
14 Rottweiler	10 y	M	III	-	-	++
15 German Shepherd	11 y	M	III	-	-	++
16 Boxer	13 y	M	III	-	-	+
17 Boxer	12 y	M	III	-	-	++

a) y: Years.

b) F: Female; FN: Neutered female; M: Male; MN: Neutered male.

c) II (malignant): low mitotic rate, less uniform cells, evidence of locally infiltrative growth or metastasis; III (malignant): high mitotic rate, cellular pleomorphism including multinucleate cells, evidence of locally infiltrative growth or metastasis reported.

d) Renal and pulmonary metastasis.

anti S-100 and anti-NSE was assessed subjectively as + (little/poorly stained), ++ (medium stained), +++ (extensive/intensively stained) and - (absent).

*Analyses statistical:* We model both tumor grade and immunohistochemical results (Cr-A and S-100) with a linear model, using a software Minitab; the output is represented in Table 1. The breed, age and sex of seventeen cases of aortic chemodectoma are showed in Table 1. Most of them were incidental findings during autopsy, six tumors were found in Boxers (35%), 3 were found in cross breed dogs (17%), different other breeds were also represented. The mean age of dogs affected by aortic body tumors was 11 years (range between 8 and 15 years), and thirteen dogs (76%) were males.

*Histological results:* All tumors had a fibrous capsule. Trabeculae of connective tissue originates from the capsule, dividing the tumor into irregular lobules (Fig. 1a). A pattern composed of chief cells (type I) and sustentacular cells (type II) surrounded by a fibrovascular stroma is the typical finding in these lobules. These cells are more numerous and are centrally distributed within the cell clusters. The cells type I are polygonal or round, with large rounded nuclei and prominent nucleoli; the cytoplasm is lightly eosinophilic, finely granular and often vacuolated. The sustentacular cells are peripherally located and spindle-shaped. The stroma surrounding these two cell lines is a mixture of nerve fibres, endothelial cells and vascular pericytes. Different grade of haemorrhage, necrosis and infiltration were observed in this neoplasia.

The histopathological grading are summarized in Table 1. In the present study the tumors fell into two groups: grade II and grade III. Eight aortic chemodectoma (47%) were of

grade II, the principal feature was the presence of a capsule with focal infiltration and a spindle cell structure. An evident distribution of cells type I and cells type II was clear. Mitoses were relatively rare in this type (two or less per high field); no metastasis were found in this group. Nine (53%) of the seventeen cases were recorded as grade III: the cells were more pleomorphic and mitoses rate was increased (four or more per high field), the most consisting data was related to big area of haemorrhages and necrosis. The lobular architecture in aortic chemodectoma grade III was lost, the cells were more pleomorphic while density was diminished. By HE it was not possible to differentiate cells type I and cells type II. In some sections we observed the infiltration of the capsule and the aortic wall. In three cases we reported the presence of vascular invasion, and in two of these dogs we found renal and pulmonary metastasis.

*Immunohistochemical results:* Staining occurred positive in all cases submitted for NSE (100%), 12 cases were considered medium intensity (70%), 3 cases high intensity (18%) and 2 cases low intensity (12%). For S-100, eight cases were negatively stained (47%) and were related to tumor undifferentiated (grade III), the remnants showed sustentacular cells positive: divided in medium intensity (23%) and low intensity (30%). Chromogranin A results, with the exception of one case, were similar to S-100 staining. Nine cases were negatively stained (53%); chief cells were positively stained in two cases medium intensity (12%), 5 low intensity (30%) and one high intensity (6%). Results are shown on Table 1. Pulmonary and renal metastasis, reported in two cases, were negative for S-100 and Chromogranin A, while NSE showed positive staining (++)

*Analyses statistical:* The regression was significant for S-

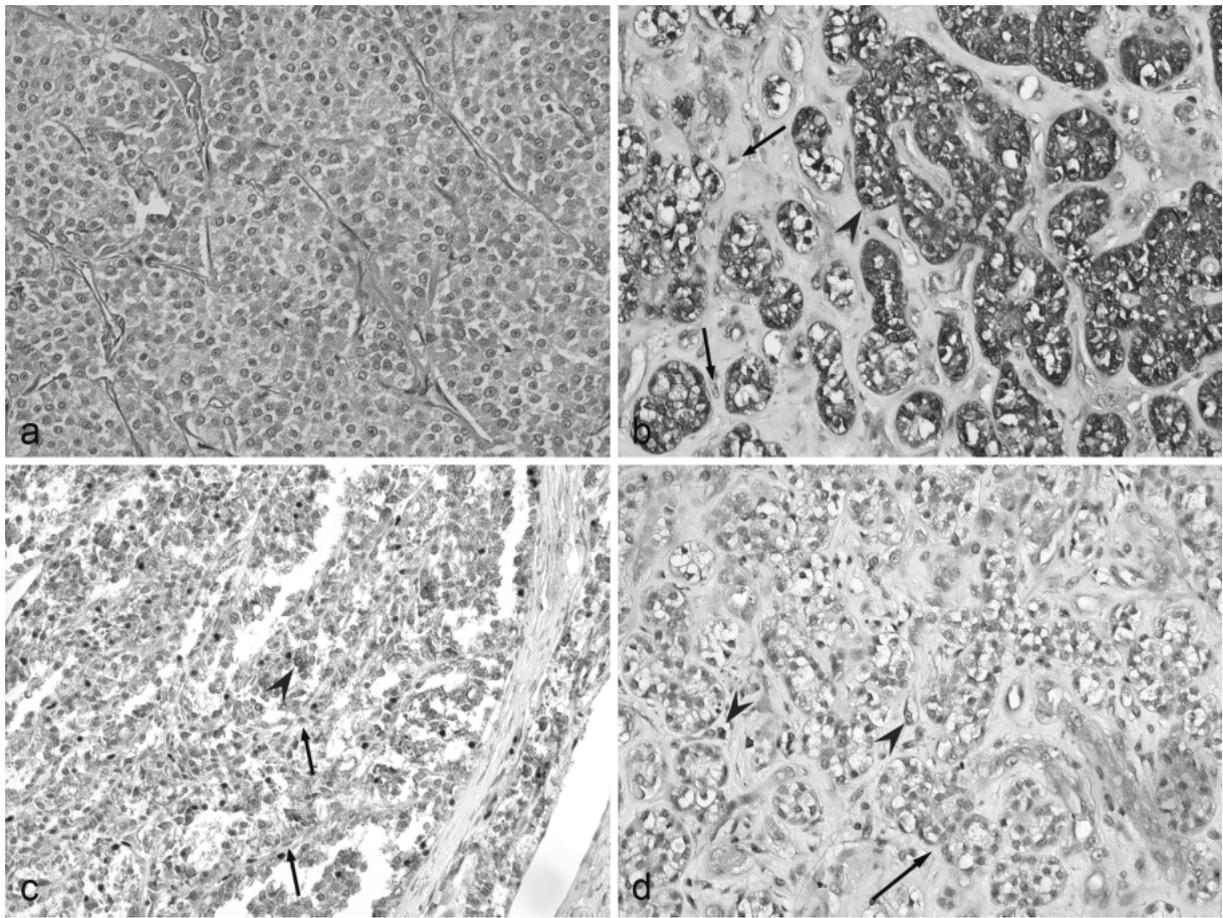


Fig. 1. a. Case No. 2. Aortic body tumor: clusters of cells Type I, polygonal cells with large rounded nuclei arranged in nests (original magnification  $\times 200$ ). HE. b. Case No. 4. Immunohistochemistry for NSE. Intensively stained. Cells type I in a lobular pattern (arrowhead) and cells type II negatively stained (arrows). (original magnification  $\times 200$ ). c. Case No. 3. Immunohistochemistry for CrA. Medium stained. Cytoplasm of chief cells brownish stained (arrowhead) and cells type II negatively stained (arrows). (original magnification  $\times 100$ ). d. Case No. 8. Immunohistochemistry for S-100. Little stained. Cells type II positively stained (arrowheads) and cells type II negatively stained (arrow). (original magnification  $\times 200$ ).

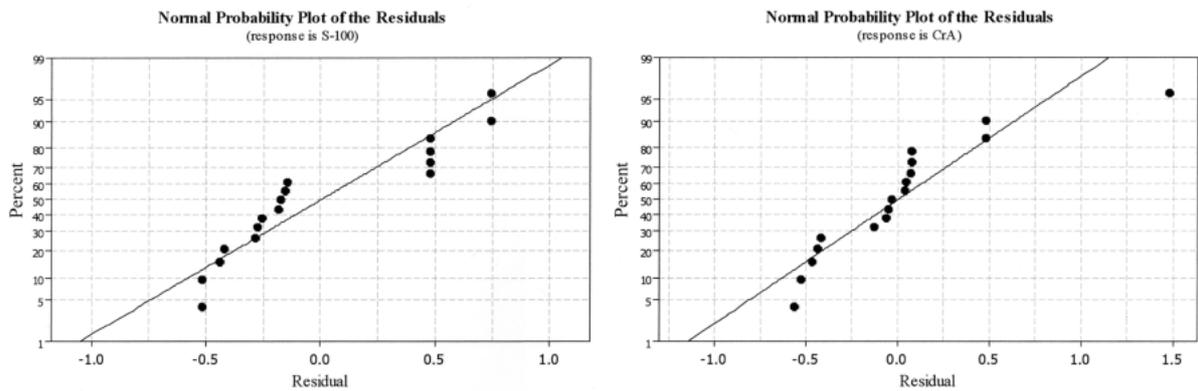


Fig. 2. Normal Probability Plot of the residuals for S-100 (left) and CrA (right).

100 and CrA antibodies; significant part of the observed variability in the data (67.5% and 71%) was explained by the regression. In Fig. 2 there are the Normal Probability Plot of the residuals, for checking the normality assumption of the errors: departures of the residuals from the normal cumulative distribution are likely if compared with the restricted number of observed points.

Results for NSE (100% positive) were considered normal for this tumor where the chief cells were expected positive [3, 7, 13], while cells Type II resulted negatively stained. Sustentacular cells showed positive nuclear staining for S-100 protein and in addition some cells showed positively staining of cytoplasmic processes, most of these cells were located at the periphery of clusters of neoplastic chief cells [1]. S-100 is commonly used in association with Chromogranin A to demonstrate the biphasic cellular makeup of paragangliomas. In human S-100 staining is diminished in malignant paragangliomas, correlated with the loss of sustentacular cells in the development of the tumor. The relationship between Type I and Type II cells is progressively lost in tumors of increasing degrees of malignancy [2, 17, 18, 23, 28].

From this report the expression of CgA and S-100 (Table 1) was consistent to the different tumor grade. This could be the most relevant data in this study. Malignant paragangliomas exhibit a variable peptide staining profile that could be attributed to altered mechanisms of biosynthesis, matter of the fact the concentration of secretory granules of chromogranin is diminished in aortic chemodectoma grade III [15, 16, 19]. Regarding the results, CrA and S-100 could be considered the most useful antibodies for the evaluation of grade of the neoplasia and a probable prognosis associated to an histological morphology. Sustentacular cell density and chief cell staining intensity were both inversely related to tumor grade (Table 1). Combinations of NSE and Chromogranin A identified chief cells in all examined cases. The immunohistochemical staining results in metastasis seem to confirm what was already demonstrated in human, where undifferentiated tumors and metastasis resulted negative to CrA and S-100.

The histopathologic criteria used to predict the biologic behaviour of paragangliomas in human are controversial. Series of criteria including the presence of mitoses, invasion of the capsule, central necrosis, invasion of the vascular spaces were used to establish a diagnosis of malignancy in this report [10, 21]. Currently, although evidence of metastasis is considered by some authors to be the criterion for establishing malignancy [18], the only presence of central necrosis, vascular invasion, and mitoses suggests an aggressive behaviour. From this report we detected that immunohistochemical techniques have the utility for diagnosis and grading of chemodectoma. Chromogranins, the structural protein of chief cells, are specific for neuroendocrine tumors [32]. The variability in staining of CgA among different cases seems to be related to the number of secretory granules as expression of differentiation of the neoplasia [6, 31]. Negative staining was observed in this report

only in tumors grade III due to degranulation of cells Type I as an indication of low synthesis of granules. Negative results for S-100 staining, specific for cells type II, were interpreted as an absence or decreased number of sustentacular cells correlated with a higher tumor grade [23]. This data was suggested with the lost of a solid structure of tumor and a lower density of cells Type II. The expression of CrA and S-100 seems to define two criteria of detection of a malignant behaviour in this neoplasia [2, 17, 18, 21, 23]. In conclusion the use of an immunohistochemical panel, in addition to routine histology, can confirm the diagnosis of chemodectoma and can express a grade of malignancy.

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