Immunohistochemical Study of the Intestinal Stromal Tumors in Dogs -7 Cases and Review of the Literature

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Abstract

Primary spindle cell neoplasms of the gastrointestinal system show considerable morphologic overlaps. Proper histological classification of these neoplasms using only the classical hematoxylin-eosin (HE) stained slides is often difficult and frequently a source of diagnosis error. Thus, the immunohistochemistry is an essential tool in the proper diagnosis and classification of such tumors.

In this study we tested and standardised an immunohistochemical protocol used for the differentiation of primary intestinal sarcomas (nonangiogenic, nonlymphogenic intestinal sarcomas) in dogs. The paper also aimed to critically review the state of art in immunohistochemistry of intestinal spindle cell neoplasms currently used in dogs.

Seven cases of primary intestinal spindle cell neoplasms were diagnosed and classified using a 6 antibody panel immunohistochemistry. Indirect immunohistochemistry staining for S100 protein, desmin, α -SMA, CD34, c-Kit and GFAP was conducted using a Leica Bond-Max auto-immunostainer.

Based on the immunoreactivity, five stromal tumours were classified as leiomyosarcomas, one fibrosarcoma and one an undifferentiated sarcoma.

In most cases of intestinal sarcomas, the proper diagnosis requests the use of immunohistochemistry. The retrospective analysis of these tumours proved that at least in one case, the HE stain was not sufficient for the proper diagnosis and classification of the tumour.

Keywords: dog, immunohistochemistry, intestinal sarcomas, intestinal stromal tumors

INTRODUCTION

In dogs, primary spindle cell neoplasms with nonangiogenic and nonlymphogenic origin include tumors with myogenic origin (leiomyoma/ leiomyosarcomas), fibrosarcoma, nerve sheath tumors and sarcomas of neurogenic origin (Heyes 2013). Although highly controversial and in disaccord with the human classification of gastrointestinal sarcomas, sometimes these tumors are identified as gastrointestinal stromal tumors (GISTs) (LaRock 1997; Heyes 2013). In this study, since no c-Kit positive "true" GIST was diagnosed, and to avoid further confusion, we will

refer to these tumors as intestinal stromal tumors (IST).

Sarcomas of the gastrointestinal system show considerable morphologic similarities, thus definitive morphologic diagnosis of these intestinal tumors, generally grouped as stromal tumors on routinely stained specimens, can be challenging (Turner 2009). In this respect, the diagnostic is highly dependent on the experience and interpretation skills of the histopathological examination.

The purpose of this study was to assess the gross, histologic and immunohistochemical

characteristics of 7 cases of canine nonangiogenic, nonlymphogenic intestinal stromal tumors which were diagnosed in the last five years in our department.

MATERIALS AND METHODS

Seven dogs corpses referred for necropsy during 2010-2015 to the Department of Pathological Anatomy, Faculty of Veterinary Medicine Cluj-Napoca, Romania, represented the material included in the study. All cases included in study were histopathologically diagnosed with primary intestinal sarcomas, excluding those with angiogenic or lymphogenic origin. A description of each case considered in this study including the breed, age and gender can be consulted in Tab. 1.

Histopathology

During necropsy the harvested specimens immersed in neutral buffered formalin (10%, pH 6.9) (Merck-Millipore, nr.100496) and fixed for minimum of 48 hours. Further tissue samples were dehydrated through successive baths of in graded isopropyl alcohol, clarified in xylene, and embedded in paraffin wax under vacuum following the laboratory routine protocol. Serial sections of 4 µm thickness were cut from the resulted paraffin-embedded tissue blocks with a rotary microtome (Leica-RM-2125) and routinely stained by hematoxylin and eosin (HE) or subjected to further immunohistochemical analysis.

Immunohistochemistry

For the immunohistochemical reaction, a Leica Bond-Max automated immunostainer (Leica Microsystems) was used. The immunohistochemistry staining was performed for S100 protein (Rabbit polyclonal, Leica Novocastra nr. PA0900), desmin (Mouse monoclonal Leica Novocastra, nr. PA0032), α-SMA (Mouse monoclonal, nr. ab76549), CD34 (Mouse, Leica Novocastra nr. PA0212), Proto-oncogene c-Kit (Rabbit polyclonal, Santa Cruz Biotechnology sc-5535) and Glial fibrillary acidic protein (Mouse monoclonal, Leica Novocastra nr. PA0026) using an antibody panel previously recommended by Frost (2003) and Hayes (2013). The immunohistochemistry protocol using a polymer-based detection system (Leica Biosystems, nr. DS9800) having as a chromogen 3,3'-Diaminobenzidine (DAB) was carried out in accordance with the protocols provided by the auto-immunostainer producer. The slides

were slightly counterstained with hematoxylin, dehydrated and mounted with a xylene compatible medium.

Bright-field images of the HE stained and for the immunolabeled, histological slides were obtained using an OlympusBX41 microscope equipped with UC30 Olympus Digital Camera and further processed by Stream Basic (Olympus Soft Imaging Solutions) and PowerPoint software.

RESULTS AND DISCUSSION

At necropsy, the tumors were characterized by unique or multinodular white-to-grey proliferative, sessile, infiltrative masses located in the ileum (3/7), jejunum (2/7) and colon (2/7). Most of tumors had a rubbery consistency and ulceration was a common observation (5/7). The age of affected dogs ranged between 5 to 13 years, with a relative balanced repartition between genders (4 females and 3 males). Regarding the affected breeds, the most affected were the boxers (3/7). It deserves to be noticed that all cases included in this study were represented by medium-sized or large bred of dogs. A description of the tumors location and size for each case are detailed in Tab. 1.

On routine histopathological examination, the tumors were moderately or densely cellular, being composed in all cases by spindle-shaped cell arranged in bundles. In some cases, without major relevance for final diagnosis, the cells were arranged in interlacing bundles with a whorl pattern. The spindle cells have in most of the cases ill-defined borders. In the case of leiomyosarcomas the cells presented moderate to abundant eosinophilic cytoplasm, with oval to elongated nuclei with blunt ends ("cigar shaped"), with a chromatin disposed in a vesicular pattern. The inflammation was frequently noticed, in most of the cases as a secondary event following ulceration (Fig. 1, image C). The fibro-vascular stroma was present in a moderate amount, being rich in collagen for the cases diagnosed as fibrosarcoma.

The histological observations were further confirmed in 6 cases by immunohistochemistry. In one case, which initially was classified as fibrosarcoma, following immunohistochemical heterogenic reaction and the presence of important malignant features, was finally classified as undifferentiated sarcoma.

In our case, the most frequent diagnosed tumor was represented by leiomyosarcomas. This observation is in accordance with the results of Heyes (2013), which found this tumor represent 32% of all type of gastrointestinal sarcomas. If we exclude the c-Kit positive GIST, in the above study the leiomyosarcomas were the most frequent diagnosis in a case of nonangiogenic, nonlymphogenic, gastrointestinal sarcomas in dogs. In addition, other studies carried out on smaller animal groups prove similar frequency of intestinal leiomyosarcomas (Patnaik 1977; Cohen 2003).

Despite this high frequency in the percentage of leiomyosarcomas from the total intestinal sarcomas, leiomyosarcomas with intestinal location are rare tumors (Brønden 2010). This can also be due to the fact that in comparison

with human oncology, the gastrointestinal tumors are more rare, in a large scale study involving 10,270 cases being diagnosed only in 1.1% of the necropsies (Patnaik 1977).

Regarding the immunohistochemical panel used by us in the study of IST, we replaced the PGP 9.5 as a marker for neurogenic derived tumors (Hayes 2013) with GFAP based on the availability of this antibody in our laboratory. The definitive morphologic diagnosis of the gastrointestinal leiomyomas can be challenging considering that spindle cell neoplasms from the digestive tract display considerable histologic overlap (Turner, 2009).

The main immunohistochemical difference between IST and GIST is the positive reactivity of the last group for Kit (CD117) (Miettinen 2006). Based on both immunophenotyping and

Fig. 1. Pathological images of two cases of Intestinal Stromal Tumors. Image A present the external aspect of a fibrosarcoma (indicated by the arrow and delimited by the circle) located in the terminal ileum in a crossbred dog. Image B present the macroscopic characteristics of a highly infiltrating jejunal leiomyosarcoma (marked by the asterisks) in a Boxer. Image C represent the histopathological characteristic of the previous leiomyosarcoma, consisting in a densely cellular tumor (black asterisk) infiltrating the intestinal mucosa (red asterisk). Image D represent a detail of C image demonstrating pleomorphic spindle cells with poorly delimited margins and eosinophilic cytoplasm. HE stain, ob x 10 for image C and x100 image D; scale bar= 400µm for image C and 40µm for image D.

ultrastructure, in its "Classification of Tumors of Domestic Animals" the World Health Organization separates both benign and malignant smooth muscle tumors from GISTs, which are composed of a mixture of cells with neurogenic and myogenic characters (Head, 2003-WHO), which probably

arise from the interstitial cells of Cajal (Miettinen, 2001). In addition to this, leiomyomas and leiomyosarcomas in dogs typically are desmin- and α -SMA-positive and CD117-negative (Bettini, 2003), while GIST prove desmin-negative.

Fig. 2. Immunohistochemical reaction of a leiomyosarcoma demonstrating intense immunoreactivity for desmin (Image A), S-100 protein (image B) and α -smooth muscle actin (image E). The tumoral cells are negative for c-kit (image F), CD34 (image D) and GFAP (image C, black asterisk). Note the positivity of immunoreaction of neurons from the myenteric plexus for GFAP (white asterisk). ob x 100 (image A)(scale bar= 40 μ m), x20 (images B, C and E) (scale bar=100 μ m) and respectively ob x10 for image E (scale bar= 200 μ m)

Tab. 1. Description of the investigated dogs

Nr.	Breed	Years, Sex	Location	Size	Diagnostic
1.	German Shepherd	5, F	Colon	ap. 3/5 cm	Leiomyosarcoma
2.	Mixed breed	7, F	Ileum	ap. 2/3	Fibrosarcoma
3.	Boxer	13, M	Jejunum	ap. 4 cm	Leiomyosarcoma
4.	Rottweiler	8, M	Colon	ap. 3 cm	Leiomyosarcoma
5.	Mixed breed	Unknown, F	Jejunum	ap. 6 cm	Leiomyosarcoma
6.	Boxer	11, M	Ileum	ap. 5/8 cm	Undifferentiated sarcoma
7.	Boxer	7, F	Ileum	ap. 3/4 cm	Leiomyosarcoma

CONCLUSION

Immunohistochemical analysis of the intestinal stromal tumors included in our study proves that proper histopathological diagnosis is enhanced by the use of immunohistochemistry. The retrospective analysis of these tumours proved that at least in one case, the HE stain was not sufficient for proper diagnosis and classification of the tumour.

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