

DOI 10.1515/pjvs-2015-0029

*Short communication*

# Immunohistochemical diagnostic of hibernoma in dog

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## Abstract

The diagnosis of hibernoma is uncommon in veterinary medicine. In this report, we present an attempt to confirm hibernoma diagnosed in dogs by applying immunohistochemical tests routinely used in human pathology i.e. antibodies specific to protein S100, protein CD31, or smooth muscle actin (SMA).

**Key words:** CD31, S100, smooth muscle actin (SMA), canine hibernoma

## Introduction

Brown adipose tissue (BAT), playing a key role in the production of heat along the pathway of endothermic adaptive thermogenesis, is manifested commonly in hibernating animals and in most species of mammals in the neonatal period. The ability of BAT to generate heat is linked with the expression of thermogenin protein (uncoupling protein 1; UCP1) localised in the inner membrane of mitochondria in brown adipocytes (Zancanaro et al. 1994).

Hibernomas are rare, benign tumors of brown adipose tissue. Diagnosed both in humans and animals, they are localised in various parts of the body and are most frequently described as a lesion developing in mature individuals (Moretti et al. 2010, Mavrogenis et al. 2011, Stuckey et al. 2013). Taking into account the appearance of the cells, character of stroma and presence in the sublayer of the spindle shaped cells, four

types of hibernoma have been distinguished: typical, myxoid, spindle cell, and lipoma-like. The most common among these is the standard type (typical hibernoma) (Chirieac et al. 2006, Moretti et al. 2010a, Mavrogenis et al. 2011, Vassos et al. 2013). In view of the very rare occurrence of hibernoma in both humans and animals, we attempted an immunohistochemical diagnosis of this neoplasm, based on data available in the literature regarding the use of anti-CD31, as an alternative method for the determination of UCP1, which was successfully used by Rosso et al. (2006) in hibernoma diagnosed in humans.

## Materials and Methods

The tumor was excised from a 12 year – old male German shepherd. An encapsulated light brown lesion was situated between the femoral muscles of

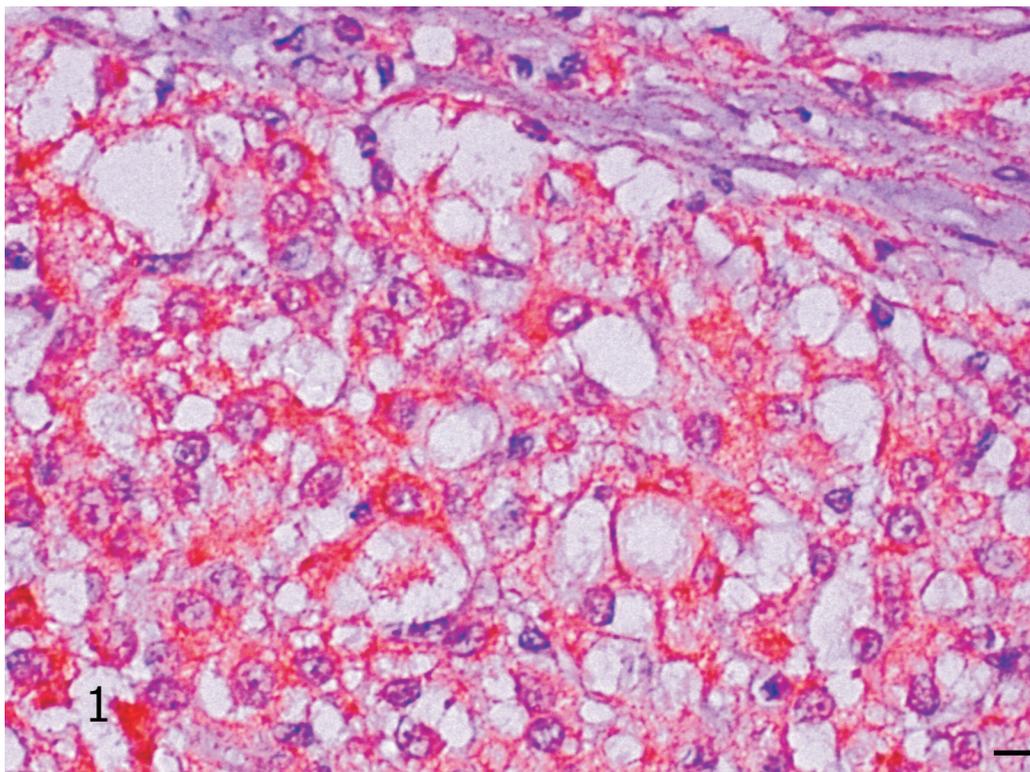


Fig. 1. Immunohistochemical staining using S100 specific antibody. Note pronounced cytoplasmic reaction in most of the cells, x400, bar=25  $\mu$ m.

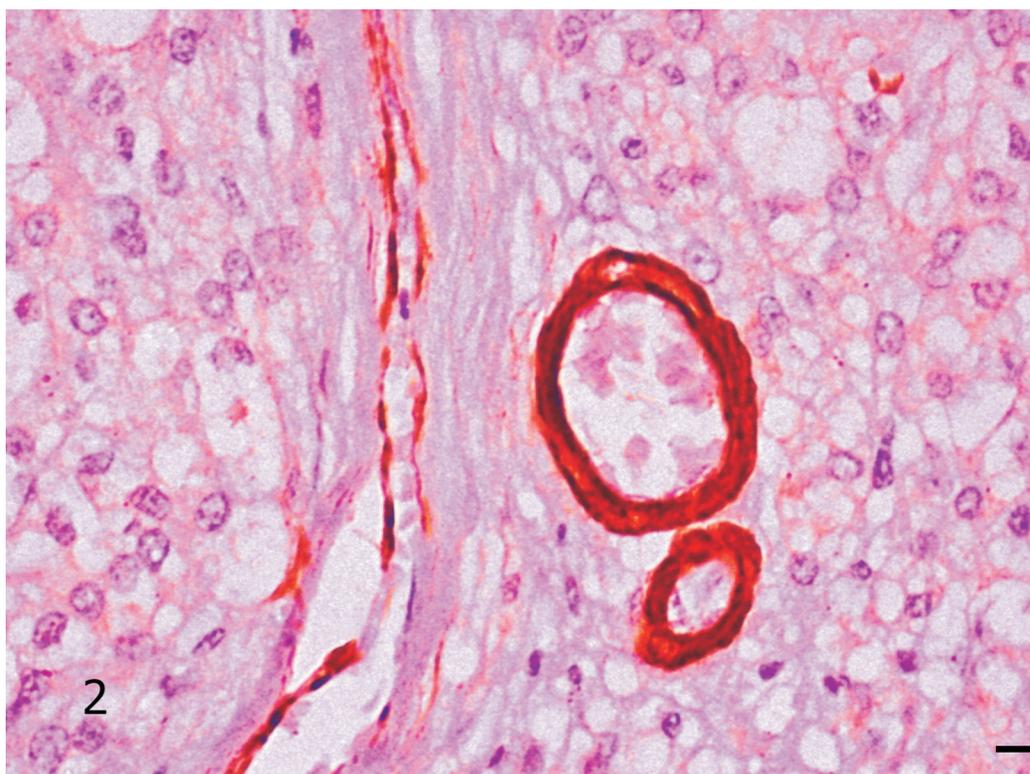


Fig. 2. Immunohistochemical staining using SMA specific antibody. Note pronounced positive reaction in vascular muscle layer and less pronounced reaction of individual connective tissue sublayer bands, x400, bar=25  $\mu$ m.

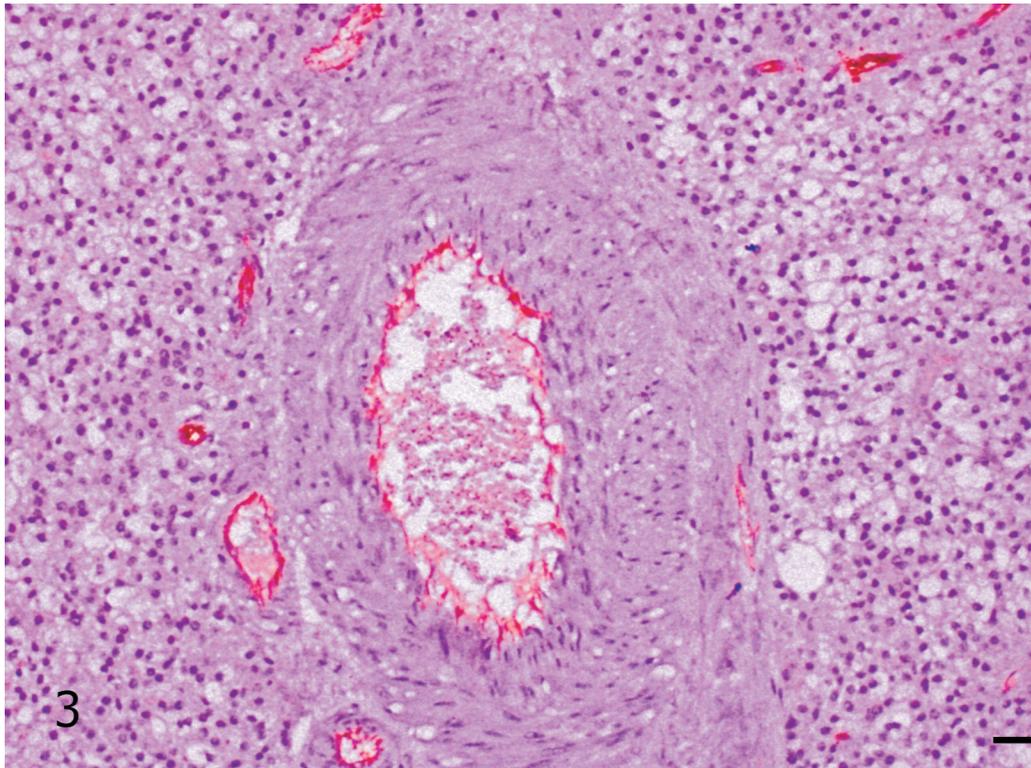


Fig. 3. Immunohistochemical staining using CD31-specific antibody. Note positive reaction in endothelial cells, x100, bar=100  $\mu$ m.

the right hind leg inducing deformation of the leg but with no manifestation of pain. The tumor tissue was fixed in formalin and embedded in paraffin for sectioning. The sections were stained with haematoxylin and eosin. After microscopic examination, tissue samples were analyzed by immunohistochemistry. Sections were stained to indicate smooth muscle actin (SMA), S100 protein and with anti – CD31 antibody based on the work of Rosso et al. (2006). The immunohistochemical analysis was performed on 4  $\mu$ m thick paraffin sections placed on silanized glass slides (DAKO® No. S 3003). Antigen retrieval was achieved by incubating the slides in citrate buffer (pH 6.0) at 97°C for 20 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. The tissue specimens were probed with a 1:800 diluted anti – S-100 protein antibody (DAKO®, Z0311), a 1:100 diluted anti – smooth muscle actin-(SMA) antibody (DAKO®, clone 1A4) and a 1:40 diluted anti – human CD31 antigen-specific antibody (DAKO®, Clone JC70A, M0823) for 20 min at room temperature, followed by incubation with peroxidase-conjugated polymer or HRP-conjugated secondary antibodies (DAKO® Real En Vision HRP). The immunohistochemical reaction was developed using 3,3-diaminobenzidine tetrahydrochloride (DAB), followed by extensive washing with water. Cell nuclei were stained with hematoxylin and specimens were dehydrated using a series of alcohol solutions.

## Results and Discussion

Histologically, the tumour was characterized by large oval or round cells with a central nucleus and a fine granular, slightly acidophilic cytoplasm with numerous small vacuoles. The nuclei were centrally located. No mitotic figures or signs of cell atypia were noted. The histological pattern corresponds to characteristics of the typical hibernoma. The immunohistochemical stainings demonstrated a positive reaction to all applied antibodies. A moderate positive reaction to S100 protein was demonstrated mainly in the cytoplasm of brown adipocytes (Fig. 1). Smooth muscle actin (SMA) was demonstrated in the muscular layer of the average and large size blood vessels localised both within the adipose tissue and in thin bands of connective tissue which separated it into lobules (Fig. 2). On the other hand, a positive reaction with CD31-specific antibody was noted in the endothelium of both small and large blood vessels and in capillaries within the tumour parenchyma (Fig. 3). Although the antibody cross-reacted with the dog tissue, the positive reaction of hibernoma cells with anti – CD31, which Rosso et al. (2006) indicated, was not confirmed in our study. The only structures which reacted with the above – mentioned antibody involved endothelial cells of blood vessels, localised both within the stroma and the parenchyma of the tumour. The

positive reaction with the S100 protein confirmed reports of other authors (Ugalde et al. 2007, Grayson et al. 2012) in which approximately 85% of all hibernoma reacted with this protein. Even though the size and arrangement of cells visible in the microscopic image could imitate large glandular cells, the presence of SMA, exclusively within the vessel wall, proved the hibernoma diagnosis and excluded the diagnosis of pleomorphic adenoma.

The described tumor type is a representative of its most frequent, typical form – the typical hibernoma.

### Acknowledgments

Publication supported by Wrocław Centre of Biotechnology, programme the Leading Notional Research Centre (KNOW) for years 2014-2018.

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