Occurrence of an Abdominal Leiomyosarcoma in the *Cyprinus carpio*: Optical and Immunohistochemical Study

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Authors' contributions

This work was carried out in collaboration between both authors. Authors LAR made the diagnosis. Author VFP managed the writing and research of literature and managed the sample processing for histopathological analysis. Both authors read and approved the final manuscript.

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ABSTRACT

A capsulated nodular neoplasm measuring 9 cm in diameter, located between the swim bladder and the kidney sample of *Cyprinus carpio*, was in laboratory. The neoplasm had a solid consistency in one sector and very adherent to the capsule. Histopathological examination of the neoplasm revealed a monotonous proliferation of spindle cells, and pleomorphic, sometimes in a vortex pattern and interspersed with collagen fibers. Cells had numerous well-oriented myofibrils giving them a deep red. The cytoplasm is eosinophilic and the nuclei are hyperchromatic located in the center with blunt or "cigar-shaped" ends. In the immunohistochemical examination, the tumor cells were positive for desmin, smooth muscle actin and K-47, the latter antibody showing significant cell proliferation. Due to histopathological and immunohistochemical findings, diagnosis was made with leiomyosarcoma.

Keywords: Immunohistochemistry; leiomyosarcoma; *Cyprinus carpio*. 

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1. INTRODUCTION

Leiomyosarcoma is called a malignant neoplasm originating from the smooth muscle. In humans leiomyosarcoma is one of the most frequent soft tissue sarcomas, with an estimated incidence ranging between 10% and 20% of all newly diagnosed soft tissue sarcomas [1]. Regarding its visceral location, uterine sarcomas are rare and constitute approximately 3% of all uterine neoplasms with leiomyosarcoma being the most common mesenchymal malignancy of the uterus [2,3]. Leiomyosarcomas, both visceral and soft tissue, have been reported in several animals [4-8].

Leiomyosarcomas in fish are rare. Among 407 neoplasms mentioned [9] of several fish species, none was leiomyosarcoma. In a recent review of neoplasms in fish, none leiomyosarcomas were found in the last twenty years [10]. This report documents a carp abdominal leiomyosarcoma studied with light microscopy and immunohistochemistry.

2. MATERIALS AND METHODS

A *Cyprinus carpio* breeder sent a 9 cm diameter whitish nodule to the Laboratory of Immunology and Pathology of Aquatic Organisms (LIPOA). This nodule was located between the swim bladder and the kidney, with a hard consistency with a thick fibroconnective capsule that separated from the neoplasia of the neighboring tissues, however in one sector the capsule appears to be infiltrated by the neoplasia. This nodule belonged to a *Cyprinus carpio* specimen weighing 980 grams and 435 cm in length (Fig. 1). The lesion was removed and then sent fixed in 10% buffered formalin. In the laboratory, the tissue was processed for histopathological study, embedded in paraffin, sectioned at 3 µm and stained with hematoxylin and eosin.

Histological sections were stained with immunohistochemical procedures, according to a modified avidin-biotin peroxidase complex technique [11]. Tissue slides were deparaffinized by rinsing with xylol and were then rehydrated with alcohols of different grades (absolute ethanol, 90%, 80%, 70%, 50%). The endogenous peroxidase activity was blocked by incubating the slides for 20 min in 0.3% H2O2 in a 5% methanol solution. After slides washed in water and in PBS/0.05%-Tween 20 solution, they were incubated in normal 1/100 serum (Vectastain Universal Elite, BC Kit, Vector), in a

![Fig. 1. Whitish nodule 9 cm in diameter, located between the swim bladder and the pronephros (K). A thick fibroconnective capsule is observed separated from the neoplaisa (short arrow), however in one sector the capsule disappears and the neoplaisa adheres to the capsule (long arrow). Bar: 2,5 cm](image)
10% PBS bovine serum albumin (BSA) solution at room temperature for 30 min in a humid chamber. After incubation, the primary anti-desmin antibody (Novocastra, Argentina), smooth muscle actin (Roche Argentina), and Ki-67 (Roche Argentina) were applied, and thereafter slides were incubated overnight at 41°C in a humid chamber. Slides were then rinsed in PBS and incubated for 7 min in a 50 mL 30.3-diaminobenzidine 1 (DAB, Sigma-Aldrich) solution containing 1% 2 PBS-BSA in 50 mL H2O2. Counterstaining was performed 3 times using hematoxylin. A human uterine leiomyosarcoma (provided by the Department of Pathology of the Piñeiro Hospital in Buenos Aires Argentina) was used as a positive control for desmin and alpha-smooth muscle actin. Human lymphoma (from the same hospital) was used also as a positive control for Ki-67. For a negative control, the primary antibody was replaced with normal non-immunized serum from the same animal species in which the primary antibody was produced [12].

3. RESULTS AND DISCUSSION

Macroscopically the nodule was surrounded by a thick fibroconnective capsule separated from the neoplaisa, however in one sector the capsule disappears and the neoplaisa adheres to the capsule. Histopathology showed a monotonous proliferation of spindle cells, pleomorphic, sometimes in a vortex pattern and interspersed with collagen fibers. Cells have numerous well-oriented myofibrils which give them a deep red. The cytoplasm is eosinophilic and the nuclei are hyperchromatic usually centrally located and has blunt or "cigar-shaped" ends. In some of these cells fused intracytoplasmic vesicles are observed. Anaplastic areas with anisokaryosis and anisocytosis were observed (Figs. 2, 3 and 4).

Immunohistochemistry was positive for desmin. Desmins are class III intermediate filaments found in muscle cells. We observed its phenotypic expression in its classic location in the cell cytoplasm. Smooth muscle actin are highly conserved proteins that participates in various types of cell motility and are ubiquitously expressed in all eukaryotic cells. We detect it in the cytoplasm as part of the cytoskeleton (Figs. 5 and 6).

The expression of the nuclear protein Ki-67 predominantly located in the G1 phase of mitosis in the perinucleolar region. We detected it in the last phases throughout the nuclear interior, being predominantly located in the nuclear matrix. This protein is strictly related to cell proliferation (Fig. 7).

Fig. 2. Monotonous proliferation of spindle cells, pleomorphic, sometimes in a vortex pattern and interspersed with collagen fibers. H-E. Bar: 200 µ
Fig. 3. Neoplastic cells are observed to have an eosinophilic cytoplasm indicated by nuclei hyperchromatic (long arrows). The nuclei are usually located in the center and have blunt or "cigar-shaped" ends (short arrow). Fused intracytoplasmic vesicles (V) are seen in some of these cells. H-E. Bar: 50 µ

Fig. 4. An anaplastic area with irregular nuclei of different sizes (anisokaryosis) shown by hyperchromatic and elongated (arrows). H-E. Bar: 50 µ
Fig. 5. Spindle-shaped neoplasm cells with positive cytoplasm for desmin. Anti-desmin. Bar: 200 µ

Fig. 6. Spindle-shaped neoplasm cells positive for alpha-smooth muscle actin. Anti-alpha-smooth muscle actin. Bar: 200 µ
In humans, leiomyosarcomas are primarily adult tumors. They are outnumbered by more common adult sarcomas such as liposarcoma and undifferentiated pleomorphic sarcoma (malignant fibrous histiocytoma). Likewise, they are lesser common than leiomyosarcomas of uterine or gastrointestinal origin. Data obtained from the collective experience with tumors in these two sizes is directly applicable to the soft tissue counterpart [13].

Histopathological diagnosis was made from the observation of spindle cells of the neoplasm and immunohistochemistry was the tool that confirmed this diagnosis [14]. The typical cell of a leiomyosarcoma is elongated and has abundant cytoplasm that varies from pink to deep red in color in sections stained with hematoxylin-eosin. The nucleus is usually centered and bimbaled or "cigar-shaped". In the same smooth muscle cells, a void is observed at one end of the nucleus, causing a slight indentation, resulting in the nucleus having a concave rather than a convex contour. In less well-differentiated tumors, the nucleus is more hyperchromatic and often loses its "central state". Giant multinucleated cells are common. In addition, depending on the degree of differentiation, the appearance of the cytoplasm varies [15]. The differentiated cells have numerous well-oriented myofibrils that are demonstrable as parallel thin longitudinally positioned deep red. In poorly differentiated cells, longitudinal striae are less numerous, poorly oriented, and therefore more difficult to identify. In some cases, the cytoplasm has a "domed" appearance as a result of shedding of myofilament material [16,17,18].

Histological variants of leiomyosarcoma are known in human pathology. Myxoid change may occur in leiomyosarcomas. When extensive, these tumors appear grossly gelatinous and are referred to as myxoid leiomyosarcoma. The so-called "inflammatory leiomyosarcoma" is a rare entity, it contains abundant inflammatory infiltrate (usually lymphocytes but occasionally neutrophils) and foci of necrosis [19]. Interestingly, although these tumors express desmin to a significant degree, they lack or only focally express other muscle markers, including a specific muscle alpha smooth muscle actin, leading to the recent suggestion that these lesions may not be a true smooth muscle structure. Rarely, leiomyosarcomas contain cells
with granular eosinophilic cytoplasm (granule cell leiomyosarcomas). This change corresponds to the presence of numerous granules that stain positively with PAS and are resistant to diastase [20,21].

The differential diagnosis of leiomyosarcomas also includes other sarcomas composed of moderately differentiated spindle cells such as fibrosarcoma, and malignant neoplasms of the peripheral nerves. Observed at low magnification, these three neoplasms are very similar [22,23]. Cytological characteristics play a very important role in the differential diagnosis. Compared with leiomyosarcoma cells, those of a fibrosarcoma tend to be conical and those of a malignant peripheral nerve. Sheath tumors are wavy, bulging, and asymmetric. Malignant peripheral nerve sheath tumors and fibrosarcomas generally do not contain glycogen; although both occasionally have cytoplasmic eosinophilia, and neither has longitudinal striae [24,25]. In addition to the above lesions, there are reactive fibroblastic lesions in the submucosa of various parenchymal organs that are often mistaken for leiomyosarcomas or even rhabdomyosarcomas [26,27]. We made the histopathological diagnosis by the spindle cell pattern of the neoplasm, but fundamentally the immunohistochemistry was a fundamental tool to confirm this diagnosis.

In humans, that are well studied, it was revealed that the aggressiveness of this neoplasia depends on pleomorphism, invasion and the presence of the number of mitoses, giant cells as a reliable criterion [28]. Regarding the degree of malignancy, some authors, both in animal and human leiomyosarcomas, predominated mitotic activity. There is a demarcation between benign and malignant tumors based on the number of mitoses. So an accurate histological diagnosis of leiomyosarcoma and its aggressiveness is possible. In the case of neoplasms with little anaplasia where the malignancy is not easy to establish carefully, some authors state that neoplasms containing more than 10 mitoses per field are leiomyosarcomas and those with fewer than this number of mitoses are well-differentiated cellular leiomyomas or benign [17,29]. Cell proliferation markers such as Ki-67 are useful to evaluate the biological aggressiveness of this neoplasm [30].

As we mentioned previously, immunohistochemistry was a useful tool to confirm the diagnosis of leiomyosarcoma. Desmin is a high molecular weight actin isoform and is expressed in smooth muscle cells, skeletal muscle, and mesenchymal cells. It is a cytoskeletal protein whose general function is regulation of cell proliferation. This marker is used for the recognition of proliferating cells and as a prognostic tool in the diagnosis of neoplasms [34]. The positive results in this case show the biological behavior of this neoplasm.

On the other hand, in antibody K-67 is a nuclear protein whose general function is regulation of cell proliferation. This marker is used for the recognition of proliferating cells and as a prognostic tool in the diagnosis of neoplasms. Smooth muscle neoplasms are rare in fish, many of them could be confused with neurolemomas currently used in immunohistochemistry to facilitate the differential diagnosis. Leiomyomas are most frequently reported including an epizootic episode in perch testis in lakes of Canada [35]. On the other hand, single leiomyosarcoma is not frequent.

As in most neoplasms, its etiology is not clear, however several neoplasms are related to viral infections. Roberts [36] reported on a series of neoplasms related to viral infection (even retrovirus), including leiomyosarcoma. A swim bladder tumor described as a leiomyosarcoma found in population of farmed Atlantic salmon in Scottish waters [37,38]. Several authors reported leiomyosarcoma induced by retrovirus [39]. It is interesting to mention that human smooth muscle tumors occur in immunosuppressed patients more frequently than in the general population. Initially reported as a complication of kidney transplantation and immunosuppression during the 1970s. These smooth muscle tumors have more recently been associated with acquired immunodeficiency syndrome (AIS) [40,41]. However, it was not until 1995 that a causal link between these tumors and Epstein-Barr virus
EBV) infection was established [42]. These tumors can be associated with either of the two EBV strains localized in soft tissue, liver, lung, and spleen. Around 50% of patients presented multiple lesions, and small tumor seedlings can often be seen adjacent to small vessels suggesting that vascular smooth muscle is a site of infection [43].

4. CONCLUSION

This short communication describes a case of abdominal leiomyosarcoma in a specimen of Cyprinus carpio. Leiomyosarcoma is an extremely rare tumor in fish. The findings in the present case indicate that leiomyosarcoma is relatively aggressive due to the expression of the nuclear protein Ki-67. Its etiopathogenic relationship with viruses was not investigated. Detailed histopathology and immunohistochemical analysis are very valuable for diagnosis and for distinguishing leiomyosarcomas from other tumors of mesenchymal cellular origin. Despite the low prevalence of leiomyosarcoma in fish, veterinary pathologists should consider leiomyosarcoma in the differential diagnosis of neoplasms composed of spindle cells.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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