

Diagnosis and surgical management of testicular seminoma in captive giant panda (*Ailuropoda melanoleuca*)

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ABSTRACT

A captive adult male giant panda (Guangzhou Chimelong Safari Park, CHINA) presented with azoospermia and enlarged left testicle. A tentative diagnosis of testicular neoplasia was made, and confirmed as testicular seminoma cases by testicular ultrasound, computed tomography (CT), testicular biopsy, and tumor marker examination findings. Based on the diagnostic results, the treatment of choice was surgical resection of the testicular tumor under general anesthesia. And the histopathological findings of the excised neoplasm were consistent with those of testicular seminoma. In addition, no tumor recurrence was observed after surgery, which indicates that our surgical and post-operative treatments were effective. The surgical treatment adopted in this case report is safe for patients and provides the best solution for the diagnosis and treatment of giant panda testicular seminoma. To our knowledge this is the first detailed report of surgical resection of testicular seminoma in the giant panda.

1. Introduction

Germ cell tumors are the most common genital tumors in male animals and usually classically manifests as a painless mass (Tourne et al., 2019). And germ cell tumors are histologically classified into leydig cell tumors (LCT), seminomas (SEM), and sertoli cell tumors (SCT) (Hohsteter et al., 2014). Seminoma occurs most commonly in young male animals and originates from transformed gonocytes (prespermatogonia and spermatogonia) (Chung & Warde, 2011). In veterinary medicine, seminoma is mainly reported in dog-related cases (Hohsteter et al., 2014). The appearance of canine seminoma is a hard, well-defined testicular mass with different necrosis and hemorrhagic lesions (Chung & Warde, 2011; Hohsteter et al., 2014). Moreover, the neoplastic cells demonstrate a characteristic tumor cell population (with distinct cell borders, vesicular nuclei, prominent nucleoli), and accompanied by lymphocyte infiltration and individual cell necrosis (Bush et al., 2011; Hohsteter et al., 2014). Surgical removal of the testis is currently the best treatment for canine testicular seminoma (Bush et al., 2011).

The giant panda (*Ailuropoda melanoleuca*) is an endangered

national first-class protected animal and one of the most adored rare species in the world, which has important research and ecological value (Martin-Wintle et al., 2019). It has been proved that the giant panda population is now stabilizing due to the efforts of the Chinese government in establishing nature reserves and the rapid development of conservation science, as well as the successful captive breeding program (Wei et al., 2015). It is worth noting that there are still very few reports on the diagnosis and treatment of giant panda diseases. Case analysis of captive giant panda is an important tool to increasing our understanding of wild giant panda diseases and improving our husbandry and management procedures for this species.

The purpose of the case reported here is to describe the clinical signs, diagnostic approach, treatment and outcome in the case of a giant panda with a testicular seminoma.

2. Case presentation

A captive adult male giant panda (8 years old, no offspring, Guangzhou Chimelong Safari Park, CHINA) presented with azoospermia and enlarged testicle. According to the breeder, semen collection was

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attempted with no success, despite the fact that the testicles were mature and normal in shape, but no treatment was commenced.

2.1. Clinical examination and diagnosis

Physical examination showed that the animal was in good condition, normal gait and no pain on examination, the appearance of the penis was normal, and the temperature of both testicles was normal. However, testicular tissue on palpation was hard, and the left testis has a longitudinal diameter of 13 cm and a transverse diameter of 10 cm, the transverse diameter of the right testis is 7.6 cm (Fig. 1). The body weight, blood pressure, and rectal temperature were 114 kg, 258 mmHg, and 37.3 °C, respectively. and hematology and biochemistry yielded no clinically significant abnormalities (Table 1-2).

Tumor markers used in evaluate of testicular cancer include α -feto-protein (AFP), carcinoembryonic antigen (CEA), β -human chorionic gonadotropin (β -HCG), and lactate dehydrogenase (LDH). Our test results showed that the serum AFP and LDH content of this patients was significantly increased (Table 3).

The result of the ultrasound examination of the left testis is shown in Fig. 2A. The left testis presented a substantial diffuse mass, with obvious blood flow signals can be seen at the edge of the mass, mixed with a large number of cystoid foci. A small amount of fluid can be seen in the scrotum, and it is highly suspected to be a manifestation of a malignant tumor of the testicular parenchyma (Thompson et al., 2020). The ultrasound examination of the right testicle showed that the echogenicity of the testicular parenchyma was increased, and there were several echogenic masses with indistinct borders inside, and significant blood flow signals were observed around the mass periphery (Fig. 2B). Furthermore, the CT images (Fig. 2C) depicted that the left testis is more than twice bigger than the right, and the outline was partially concave and convex. The texture was uneven, showing multiple dark areas and shadows, with cystic boundaries, and the density is slightly higher than that of normal tissues. There is a high-density calcification lesions in the right testis. The pathological examination of the pathological testis fine-needle aspiration biopsies (FNA) showed a large number of granulocytes of various shapes, and abnormal cells such as spindles and stars. And the nucleus is enlarged, deformed, chromatin is unevenly distributed, and high nucleus to cytoplasmic ratio (Fig. 3A). H&E staining showed that the mass was composed of round cells with uniform morphology. The cytoplasm of the cells was transparent and rich in glycogen. The nucleus was large and regular, with one or more eosinophilic nucleoli (Fig. 3B).

Based on the combination of the findings above the final diagnosis was presumptive seminoma of the left testis.

2.2. Treatment and outcome

After discussion, the surgical procedure was performed following the advice of clinicians. Prior to surgery, to prevent hypersalivation, atropine sulfate (Shanghai Tongren pharmaceutical CO., LTD, China) at

Table 1
Results of blood routine tests.

	Result	Tendency
White Blood Cells ($\times 10^9/L$)	6.5	–
Lymphocyte (%)	17.7	–
Monocyte (%)	9.6	–
Neutrophil (%)	72.6	–
Red Blood Cells ($\times 10^9/L$)	10.06	–
Hemoglobin (g/L)	19.6	–
Hematocrit (%)	44	–

Table 2
Results of serum biochemical tests.

	Result	Tendency
Creatinine ($\mu\text{mol/L}$)	177.0	–
Glucose (mmol/L)	3.40	–
Uric acid ($\mu\text{mol/L}$)	81.80	–
Total protein (g/L)	64.20	–
Albumin (g/L)	36.0	–
Total bilirubin ($\mu\text{mol/L}$)	3.90	–
Aspartate aminotransferase (U/L)	131.70	–
Alanine aminotransferase (U/L)	70.80	–
Creatine kinase (U/L)	178.7	–

Table 3
Tumor marker test.

	Result	Reference range	Tendency	unit
chorionic gonadotropin (β -HCG)	< 0.5		–	IU/mL
alpha fetoprotein (AFP)	28.5	0–10	↑	ng/mL
carcinoembryonic antigen (CEA)	< 0.6	0–6	–	ng/mL
lactate dehydrogenase (LDH)	1420	109–245	↑	U/L

0.02 mg/kg was administered in the musculature of the right thoracic limb. Anesthesia was induced with 8.7 mg/kg of ketamine hydrochloride (Jiangsu Zhongmu Beikang Pharmaceutical Co., Ltd, China) intramuscularly (IM). Then, the giant panda was positioned in dorsal recumbency and anesthetized by continuous administration of isoflurane (RWD Life Science Co., Ltd, China) at a concentration of 2% in oxygen via mask. Then the bilateral orchietomy was performed and the testicles were submitted for histological analysis. The resected right testis weighed 240 g, and the left testis weighed 580 g, with a good capsule appearance (Fig. 4). Ceftriaxone (3 g IV, Qilu Pharmaceutical Co., Ltd, China) was administered during the surgery and oral amoxicillin capsules 1500 mg. Postoperative management included oral amoxicillin capsules (Qilu Pharmaceutical Co., Ltd, China) 1500 mg, once a day for one weeks; external application of Yunnan Baiyao powder (Yunnan Baiyao Group Co., Ltd, China) and mupirocin ointment (Sino-



Fig. 1. The giant panda presenting a marked left testicular enlargement.

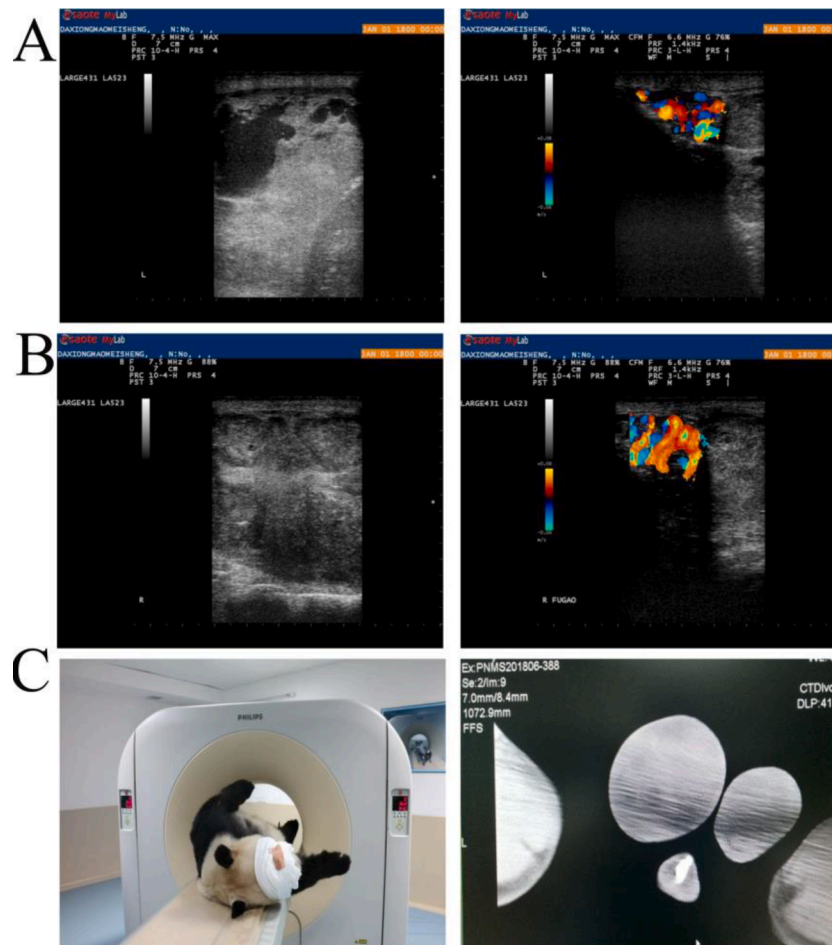


Fig. 2. (A) Ultrasonogram findings for a giant panda with enlarged left testicle. (B) Ultrasonogram findings of the right testicle. (C) Computed tomography image of the testicle.

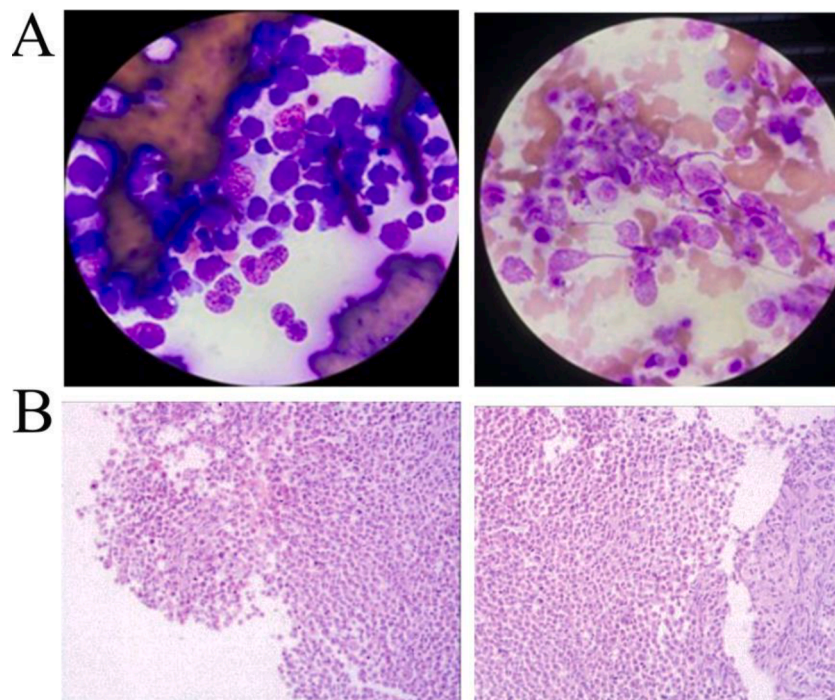


Fig. 3. Biopsy pathological examination of testicular tissues. (A) Diff Quick stain, 400 X. (B) H&E stain.



Fig. 4. Clinical manifestations and surgical management of testicular seminoma in giant panda.

US Tianjin SmithKline Pharmaceutical Co., Ltd., China) at the skin sutures; and preventing giant panda from scratching the wound. The wound healed well 2 weeks after the operation, and there was no tumor metastasis temporarily.

The excised left testicle showed adipose-like lesions, and the excised right testicle showed a gray-yellow caseous necrosis in the center (Fig. 5). Histologically, the right testicle showed proliferation of the parenchymal cells in the seminiferous tubules (Fig. 5A). The normal tissue structure of the left testicle was lost and eroded by a tumor, with hyperchromatic cytoplasm and increased nuclear chromatin. Pathologic mitotic phases were found in tumor cells, and necrotic lesions of different sizes were distributed in tumor tissues (Fig. 5B). On the basis of macro and histopathologic examination results, the tumor was

diagnosed as seminoma.

3. Discussion and conclusion

Giant panda is an endangered mammalian species (Kang & Li, 2018). Understanding and addressing the disease threat of giant pandas is essential to the conservation of this species. In most previous cases, gastrointestinal diseases, parasites and viral infections were the main causes of death of giant pandas (Wang et al., 2018; Zhao et al., 2017, 2021). However, there is little information about the diagnosis, treatment, and rehabilitation monitoring of the giant panda's reproductive organ diseases. Therefore, we chose bilateral orchidectomy and described these procedures in detail; our methodologies and results will

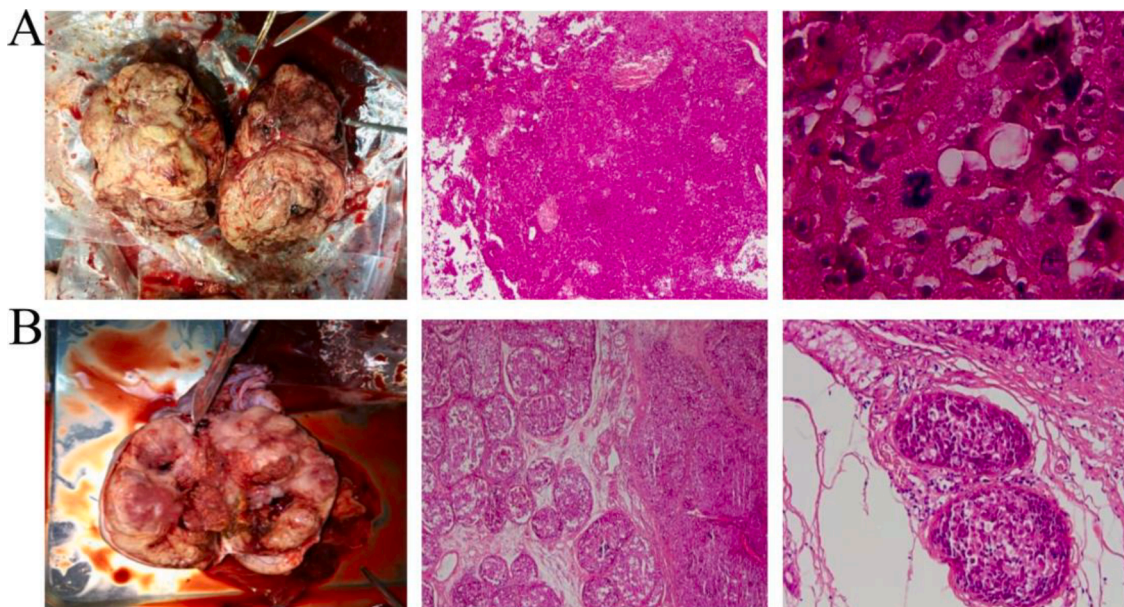


Fig. 5. Pathological features of testicular seminoma in giant panda. (A) Left testicle. (B) Right testicle.

provide a great reference for wildlife veterinarians and related researchers.

According to the literature, about half of testicular cancers are seminoma, which is derived from the neoplastic transformation of germ cells (Bush et al., 2011; Schmoll et al., 2009). At gross pathologic analysis, a typical seminoma is usually a well-defined mass, brown to light yellow, solid and fleshy (Chung & Warde, 2011). At histologic assessment, seminoma showed nested and flaky cells, with obvious cell boundaries and mild to marked basophilic cytoplasm (Bush et al., 2011). There was marked anisocytosis and anisokaryosis with prevalent karyomegalic cells (Bush et al., 2011). The testicular pathological changes observed in this case report have pathological features consistent with seminoma. In addition, over-expressed AFP and LDH were also detected in the serum (Motzer et al., 2015). AFP is a tumor antigen that is a marker for clinical tumor detection. The level of AFP in spermatogonia should be normal, and only expressed in large amounts during embryonic development and in adult tumor cells (Kandori et al., 2017; Motzer et al., 2015). LDH is a nonspecific marker of overall tumor burden (Junuzovic et al., 2011). In clinical examination, in addition to detecting the biomarkers of testicular tumors in the serum, ultrasonography, computed tomography, and biopsy can also be used for auxiliary diagnosis (Hohsteter et al., 2014; Marko et al., 2017; Zouhair et al., 2010). Studies have found that the ultrasonographic results of testicular seminoma showed hypoechoicity compared with the background testis (Marko et al., 2017). And they are often homogeneous and may be lobulated or multi-nodular (Marko et al., 2017). In the present case, we also used the above-mentioned inspection methods to detect the diseased testis, and finally diagnosed as seminoma of the left testis based on the test results.

Previous studies have shown that for testicular seminoma, the standard treatment is orchiectomy, and testicular tumors generally have a good prognosis (Yerram et al., 2017). Therefore, this case also chose the treatment method of surgical removal of testicular tumors under general anesthesia. The giant panda testicular tumor resection implemented in this case report can be successfully applied in veterinary clinics. The surgical treatment adopted in this case report is safe for patients and provides the best solution for the diagnosis and treatment of giant panda testicular seminoma. Additionally, it is the first description of a successful treatment with giant panda testicular seminoma.

Ethical statement

All necessary steps taken in this report have been carried out in accordance with high standards of animal care (veterinary care).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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