



2024. Volume 15, No. 3. P. 113-135

Received: 09.05.2024 Revised: 17.07.2024 Accepted: 15.08.2024

DOI: 10.31548/veterinary3.2024.113

UDC 616-071:616-006:616.411

Clinical and morphological analysis of splenic neoplasms

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Abstract. The relevance of this study arises from the pressing need for a deeper investigation into the morphological aspects of identifying focal and localised lesions in the spleen. The spleen performs several vital functions, including immune, haematological, storage, metabolic,

Suggested Citation:

Marunchyn, A., Kovalova, A., Marcin, P., Tereshchenko, K., & Honcharenko, M. (2024). Clinical and morphological analysis of splenic neoplasms. *Ukrainian Journal of Veterinary Sciences*, 15(3), 113-135. doi: 10.31548/veterinary3.2024.113.

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haemostatic, hormonal, and antitlastic roles, among others. Therefore, this study aimed to determine the clinical characteristics of splenic tumour lesions and their structural changes, as well as to identify the macroscopic appearance of the organ post-splenectomy. In diagnosing splenic diseases, a key instrumental method was employed – ultrasound examination with Doppler imaging – which enabled comprehensive investigation, analysis, and characterisation of parenchymal pathologies. Animals underwent clinical examinations during routine health checks or upon presentation of symptoms. When a splenic neoplasm was detected, surgical treatment was carried out, specifically through an open laparotomy and splenectomy. The surgery was performed under combined general anaesthesia (using both non-inhalational and inhalational anaesthetics). The intraoperatively collected material from the affected organ was subjected to histological examination. It was found that the pathomorphosis of splenic lesions varies, and the author's pathological and histological studies highlight the key morphological characteristics of the neoplasms. Accordingly, any splenic neoplasm can pose a life-threatening condition for several reasons: organ rupture and haemorrhage, organ torsion, and malignancy, among others. The findings are crucial for verifying splenic pathology, enabling the establishment of a definitive clinical diagnosis, facilitating treatment of the animal, and extending its lifespan. The practical significance of the results lies in identifying the need for comprehensive clinical and morphological investigations in diagnosing splenic diseases, as well as examining histological preparations for cellular and tissue changes. In veterinary medicine, the method of surgical treatment for splenic lesions in animals may be a subject of debate. In contrast, in human medicine, established protocols exist for performing organ-destructive operations such as splenectomy (by open or laparoscopic approach), organ-preserving procedures like splenic resection or splenectomy with autotransplantation of splenic tissue into the greater omentum (by open or laparoscopic approach), and percutaneous punctures for splenic cysts

Keywords: ultrasound examination; lymphogranulomatosis; myelolipoma; structural changes; laparotomy; endothelial cells; non-Hodgkin lymphoma

Introduction

According to the classification provided by A.Y Mazurkevich *et al.* (2023), mesenchymal-origin neoplasms are categorised into tumours of fibrous, nervous, adipose, muscular, and synovial tissues, as well as of vascular origin and myxomas. In dogs, fibromas, fibrosarcomas, leiomyomas, haemangiomas, and malignant fibrous histiocytomas are more common. In cats, fibrosarcoma predominates, often associated with the feline fibrosarcoma virus. Vascular-origin tumours are relatively frequently diagnosed in dogs, ranking 1st to 3rd among soft tissue neoplasms. The average age of affected animals is 8-9 years, with an almost equal

gender ratio. These tumours typically localise in the neck, torso, and limbs (with the pelvic limbs being affected 2.6 times more often than the thoracic limbs). The spleen, like soft tissues, is also frequently affected. This is a heterogeneous group of tumours. According to the classification from the 1990s, the following are distinguished: angioma, angiosarcoma, epithelioid haemangiosarcoma, spindle-cell haemangiosarcoma, malignant endovascular papillary angioendothelioma (Dabska tumour), haemangiopericytoma, and retiform haemangiosarcoma. A distinctive feature of the latter is the presence of long, branching vessels

lined by monomorphic endothelial cells and pronounced lymphoid infiltrates. Cytological differentiation of these tumours is not always possible. The vascular origin of the tumour is indicated by the presence of blood vessels in the material for examination.

J.M.M. Rodriguez *et al.* (2020) conducted histological and immunohistochemical investigations of a haemangioma found in the spleen of a grey wolf (*Canis lupus lupus*) and compared the results with those obtained in dogs. The authors note that there are relatively few published reports on the pathology of the spleen in carnivores. The results show similar characteristics to dogs, in which neoplastic cells express vimentin, von Willebrand factor, antibodies to smooth muscle actin and endothelial growth factor. Special staining with toluidine blue demonstrated a moderate increase in the number of mast cells infiltrating the tumour. The study focuses on this particular feature of the neoplasms.

In the studies of M.V. Utechenko (2022) and M. Quinci *et al.* (2020), it was highlighted that one of the reliable methods for differentiating focal splenic lesions is the use of ultrasonography results, microscopy, and imaging of neoplastic processes, which assist in determining treatment strategies and prognosis. A review of the specialised literature reveals differing views on the functional role of the spleen in mammals. Some researchers do not classify this organ as essential for survival, although they do not deny its significance for the proper functioning of the body. When pathological processes affect the spleen, splenomegaly is often observed, which may result from the acute or chronic impact of a pathological factor.

S. Griffin (2021) highlights the importance of ultrasound examination of the abdominal organs, especially when splenic neoplasms are discovered incidentally. The author emphasised that diagnostic accuracy is quite high when performed by a skilled operator. However, all detected splenic neoplasms, whether localised or

diffuse, are indications for surgical intervention.

G. Corvera *et al.* (2024) demonstrated the critical role of the spleen in the body, performing at least three essential functions: eliminating foreign particles and bacteria from the blood; participating in immune responses; and producing blood cells when bone marrow haematopoiesis is suppressed, as seen in myeloid metaplasia of the spleen, which occurs as a restoration of the haematopoietic function originally active during embryogenesis.

The spleen also plays a role in stress adaptation. Through the activity of beta-adrenoreceptors, its capsule contracts, releasing stored erythrocytes into the bloodstream, thereby increasing the blood's oxygen-carrying capacity. In some animal species, the spleen functions solely as an organ of lymphopoiesis. During embryogenesis, it acts as a mixed lymphomyeloid structure and regulates the cytological composition of the blood.

N. Kim *et al.* (2022) found that following a splenectomy, the body's resistance to bacterial and viral agents decreases, leading to complex changes in both the humoral and cellular branches of the immune system. This includes a reduction in the clearance of particulate antigens, inhibition of phagocytic activity, and suppression of antibody production in response to non-specific antigens. Alterations in the spleen's filtration function result in hypercoagulopathy and thrombocytosis, with an increased risk of excessive thrombus formation post-surgery, which is explained by elevated levels of TNF- α and interleukin-6 in the blood, as well as overproduction of platelet antibodies. The immune response is also weakened, and IgG catabolism increases. Post-splenectomy immune disorders involve reduced levels of IgG1 and IgG4 fractions, as well as lower concentrations of fibronectin and IgM, accompanied by impaired functional ability of neutrophil granulocytes, monocyte cytotoxicity, and shifts in lymphocyte population ratios.

Consequently, the authors emphasise the importance of differential diagnosis in the study of splenic neoplasms.

Based on previous research reflected in specialised publications, this study aimed to conduct post-operative histological studies of splenic neoplasms detected by ultrasound.

Literature Review

In their study, T. Fossum *et al.* (2019) demonstrated that the spleen plays a crucial role in animals by producing blood cells, which, under conditions of myeloid metaplasia, support the restoration of haematopoiesis. A. Biriş *et al.* (2019) noted that splenic tumours are common in dogs and are often diagnosed during routine screening using ultrasound examination of the abdominal organs, as well as in patients showing clinical symptoms of the disease. Statistical data were collected from April 2007 to June 2018, indicating that splenic tumours accounted for 6% (97 cases) of the total (1,629 cases) neoplasms examined. Histologically, malignant tumours were found in 89% of cases, benign tumours in 11%, with haemangiosarcoma diagnosed in 75% and splenic lymphoma in 22%. Of the 97 splenic tumours included in this research, 64 were diagnosed as primary haemangiosarcoma, with 56% exhibiting metastases in various organs of the body.

According to the research of J.M. Connors *et al.* (2020), Hodgkin's lymphoma is classified as a B-cell lymphoma. The clonal proliferation of so-called Hodgkin and Reed-Sternberg cells, which belong to the B-cell population and are surrounded by reactive cells, occurs primarily in the lymph nodes before spreading to the spleen and other organs. The aetiology of the disease remains unknown. In human medicine, the condition is predominantly observed in adolescents and young adults, although it can also affect the elderly. Diagnosis is based on histological and immunohistochemical exam-

ination of tissue following a biopsy. Morphological changes in the tissue and the antigen expression profile allow for the classification of the lymphoma subtype (nodular sclerosis, mixed cellularity, lymphocyte-depleted, or lymphocyte-rich lymphoma). A thorough understanding of the pathology is essential for determining the approach to multimodal therapy, including radiation and multi-agent chemotherapy.

G. Corvera *et al.* (2024) determined that in dogs, the spleen functions as a secondary lymphoid organ where both neoplastic and non-tumorous nodular formations of varying sizes can develop. The research aimed to characterise several focal lesions and identify risk factors specifically associated with tumour development. A total of 507 histological reports were examined. Hemangiosarcoma and hyperplasia were most commonly observed in Cocker Spaniels, German Shepherds, and Labrador Retrievers. The collected data were analysed using descriptive statistics and multiple logistic regression. Similarly, E.J. Herman *et al.* (2019) reported that hemangiosarcoma is a prevalent tumour in geriatric dogs. To confirm the pathohistological changes, it is necessary to examine the entire organ. M.A. Griffin *et al.* (2021) suggested that hemangiosarcoma in dogs and cats originates from pluripotent bone marrow with a multifactorial pathogenesis. In dogs, the disease is recorded more frequently than in cats. According to the authors' studies, affected animals have an unfavourable prognosis with rapid metastasis. The primary treatment method is complete splenectomy combined with chemotherapy based on anthracyclines. J.M. Rozolen *et al.* (2021) reported that splenic hemangiosarcoma is a malignant tumour arising from endothelial cells and predominantly affects middle-aged and older dogs. The tumour leads to extensive necrosis and haemorrhages within the organ's parenchyma. Their study utilised 53 samples of splenic tumour

tissue from dogs that had been previously diagnosed through histopathological examination, which were fixed in formalin and embedded in paraffin. The expression levels of Claudin-5, PSMA, and Ki67 proteins were evaluated using immunohistochemistry, while gene expression was assessed through quantitative polymerase chain reaction. Overexpression of Claudin-5 was observed in patients with metastases and those with stage III tumours compared to patients with stages I and II. The findings indicated that the expression of Claudin-5 protein is associated with the metastatic process, whereas the expression of the PSMA gene and Ki67 protein correlates with survival time.

F.J. Leyva *et al.* (2018) histopathologically investigated the characteristics of concurrent neoplasms of the spleen and liver in dogs that underwent complete splenectomy and biopsy. The study included 125 patients (dogs), of which 27% (34/125) showed no signs of malignant neoplasms, while 48% (60/125) had malignant neoplasms of both the liver and spleen. Notably, 56 of these 60 dogs presented with identical malignant neoplasms in both organs.

The relevance of splenectomy in cases of diffuse splenic lesions was discussed by A. Ampese *et al.* (2022). The authors noted that myelolipoma of the spleen is a benign neoplasm composed of adipocytes and haematopoietic tissues. In comparison to the incidence of tumours such as haemangiosarcoma, fibrosarcoma, haemangioma, and lymphoma, myelolipoma is relatively rare. Typically, the disease remains asymptomatic in animals. In some cases, symptoms such as vomiting, abdominal distension, and signs of pain were observed. The presence of a neoplasm was confirmed through ultrasound diagnostics. In cases of significant neoplasms and splenomegaly, adjacent internal organs, including the liver, pancreas, and kidneys, may become compressed. The surgical treatment for myelolipoma involves total sple-

nectomy. L. Cassaro *et al.* (2021) reported that myelolipoma can occur in both dogs and cats and may develop in various organs and tissues. A clinical case of splenic myelolipoma in a dog was described, detailing its clinical-pathological status and ultrasound examination findings. Ultrasound imaging revealed splenomegaly with formations along the entire length of the parenchyma, exhibiting mixed echogenicity and heterogeneous echostructure, likely due to the mixing of tumour components with adipose tissue. Macroscopically, the spleen appeared enlarged, with the presence of irregularly shaped nodules on the surface of the organ, which were red with small yellowish areas. Microscopically, a well-defined encapsulated formation was observed, consisting of differentiated neoplastic adipose tissue with islands of haematopoietic elements in varying proportions. J.A. Nitz *et al.* (2020) described a clinical case of splenic myelolipoma in a human patient. Ultrasound imaging, along with computed tomography, identified a splenic neoplasm with characteristics consistent with adipose tissue. Following a total splenectomy, a pathological examination of the organ was conducted, confirming the diagnosis. The authors emphasised that the differential diagnosis of splenic neoplasms containing adipose tissue, as seen on computed tomography, ranges from benign tumours to malignant mesenchymal origins.

J.L. Kutok & C.D.M. Fletcher (2005) established the existence of both benign and malignant tumours of the spleen. Benign tumour types include lymphangiomas, haemangiomas, and endothelial tumours, with osteomas, chondromas, and fibromas being exceedingly rare. Malignant tumours encompass specific types of sarcomas, such as lymphosarcoma, fibrosarcoma, angiosarcoma, and reticulosarcoma, among others. The authors focused on the differential diagnosis of vascular tumours of the spleen in their contemporary context.

The splenic angiomata were investigated by P.P. Arcuri *et al.* (2022). The authors noted that primary angiomata of the spleen are generally benign; however, instances of malignancy have been reported. They cited a case study involving a patient who underwent diagnostic investigations using both computed tomography with contrast agents and magnetic resonance imaging.

P.A. Cole (2012) and K. O'Byrne & G. Hosgood (2019) demonstrated that various diseases can lead to neoplasms of the spleen. Their research was based on ultrasonography and computed tomography. The comparisons were conducted across different breed sizes: small, medium, and large. They found that the prevalence of malignant and benign tumours did not significantly differ; however, small-breed dogs were more likely to have benign neoplasms. This information is important to consider during initial consultations with pet owners.

In summary, the literature indicates that splenic neoplasms are relatively common in animals. Pre-operative clinical examinations and post-operative histological studies are crucial for accurate diagnosis. Splenic lesions can be both benign and malignant.

Materials and Methods

The study was conducted between 2020 and 2023 at the "Universal Veterinary Care" clinic and the pathology department of the "Universal Clinic Oberih" medical centre in Kyiv. Thirteen illustrative clinical cases of splenic neoplasia were documented. All experimental procedures involving animals were conducted following the fundamental principles of bioethics, following the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986) and the Procedure for Conducting Research and Experiments on Animals by Scientific Institutions (2012).

In particular, the results of surgical treatment for various species of animals are presented: an 11-year-old female Dachshund; an 11-year-old female Yorkshire Terrier; an 11-year-old female Jack Russell Terrier; a 10-year-old female French Bulldog; a 10-year-old male Yorkshire Terrier; a 10-year-old male Jack Russell Terrier; a 10-year-old male mixed-breed dog; a 10-year-old female Yorkshire Terrier; a 9-year-old female Pug; an 8-year-old male Yorkshire Terrier; a 3-year-old male cheetah (*Acinonyx jubatus*); a 6-year-old male cheetah (*Acinonyx jubatus*); and a 6-year-old female cheetah (*Acinonyx jubatus*).

All affected animals underwent clinical and instrumental examinations, including an ultrasound of the abdominal organs using the MyLabOneVet veterinary scanner (Esaote). The detected neoplasm was measured and characterised in terms of the echogenicity of the affected area, its relation to surrounding organs, and the blood circulation in the affected region was assessed using Doppler ultrasound.

The protocol for general anaesthesia used was as follows: an anaesthetic mixture was administered intramuscularly, consisting of Domitor Vet (medetomidine hydrochloride 1 mg/mL) at a dose of 0.01 mg/kg and Butomidor (butorphanol) at 0.1 mg/kg of the animal's body weight (Orion Pharma, Finland). Surgical intervention was conducted under isoflurane endotracheal anaesthesia (Iso-Vet, isoflurane 1000 mg/g, United Kingdom). For this purpose, the Komesaroff small animal closed circuit anaesthetic machine MINI-KOMV (Australia) was used, along with endotracheal tubes with a cuff.

The examination of animals under anaesthesia was conducted using clinical methods and a device that monitored pulse rate, respiratory rate, blood oxygen saturation, rectal body temperature, and arterial blood pressure (via an automatic cuff placed on the brachial area of the limb) known as InnoCare-Vet (Hungary).

The surgical procedure involved an open laparotomy of the upper midline abdominal wall along the linea alba. Small bleeding vessels were coagulated during the operation using bipolar forceps connected to the device EXBA-350M/120B “Nadiya-2” (Ukraine). Ligation was applied to the larger vessels supplying the spleen. The surgical wound was closed in layers, primarily using simple interrupted sutures. Absorbable surgical suture material, Syntil No. 3 and 4, was employed for ligatures, soft tissue of the abdominal wall, and skin.

Subsequently, in pursuance of the aim, tissue samples were taken from the pathologically altered areas of the spleen and fixed in 10% neutral formalin for further investigation.

Subsequently, histotechnical processing of the material was performed using the STP120 Microm / Thermo Scientific (Germany) tissue processing machine of carousel type, which involved dehydration in a series of ascending concentrations of alcohol, followed by embedding in paraffin. Paraffin blocks were prepared using the EC350 Microm/Thermo Scientific (Germany) tissue embedding centre. From these blocks, serial sections were produced with a thickness of 3–5 μm using the HM340E Microm/Thermo Scientific (Germany) rotary microtome, which was additionally equipped with the STS Microm/Thermo Scientific (Germany) transfer system for sections. The samples were placed on standard glass slides, followed by deparaffinisation and histological staining in the HMS 740 Microm (Germany) staining machine. The staining protocol involved haematoxylin (Harris Haematoxylin Accified series S, Epre-dia, Spain)/ eosin (Eosin Y Alcoholic series R/ Epre-dia, Spain). Upon completion of the staining process, the tissue samples were mounted under glass using the mounting medium Micromount/DiaPath (Italy). Subsequently, the histological preparations were examined using light microscopy with an Olympus BX 46 micro-

scope (Japan) equipped with an Olympus SC 50 camera (Japan), with sequential observations at magnifications of $\times 4$, $\times 10$, $\times 20$, and $\times 40$ (with a $\times 10$ ocular).

Results and Discussion

According to patient medical histories, it was established that the symptoms of splenic neoplasms may not manifest clinically, or the disease could follow a subclinical course. In cases of malignant tumour development, the symptoms may be more pronounced, particularly with rapid metastasis. Clinical cases of 13 patients diagnosed with splenic neoplasms through ultrasonographic examination, followed by splenectomy and morphological studies, are presented.

Non-Hodgkin lymphoma. Dog, Dachs-hund, 11-year-old, female.

Medical history: Clinical symptoms included abdominal wall pain, with noted tension in the area. During clinical examination using ultrasound of the abdominal organs, the following findings were recorded: the spleen was not enlarged, crescent-shaped, with smooth contours and clear boundaries. The echogenicity was increased, and the structure was homogeneous. A mass, measuring 1.2×1.1 cm, was visualised in the middle third of the spleen. It had a homogeneous hypoechoic internal structure with irregular and unclear borders, and blood flow to the mass was not enhanced (Fig. 1). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, palpably not compacted, with a 1.2×1.1 cm nodular growth of light grey tissue with unclear margins, penetrating into the normal splenic parenchyma (Fig. 2). Morphological changes: The splenic parenchyma was histologically normal, with typical white and red pulp organoid structure. Tumour growth focus: a focus of proliferating lymphoid tissue forming atypical follicular structures,

composed of neoplastic cells of the lymphoid series. These cells were of medium size, with well-defined nucleoli, and sporadic figures of

mitotic divisions were observed (Fig. 3). This histological pattern is indicative of non-Hodgkin lymphoma.

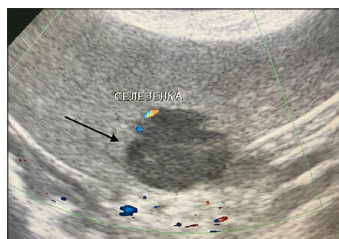


Figure 1. Ultrasound image of the neoplasm

Note: hypoechoic neoplasm measuring 1.2×1.1 cm

Source: authors' material



Figure 2. Intraoperative photo of the splenic neoplasm

Note: neoplasm localisation

Source: authors' material

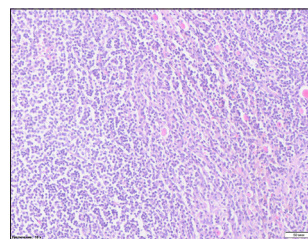


Figure 3. Atypical lymphoid proliferation in splenic tissue

Note: Haematoxylin-eosin staining, magnification ×100

Source: authors' material

Cavernous haemangioma. Dog, Yorkshire Terrier, 11-years-old, female.

Medical history: The splenic tumour was discovered incidentally during a dispensary examination. No clinical symptoms of the disease were observed. **Ultrasound examination:** The spleen was not enlarged, with a typical crescent shape, smooth borders, and well-defined edges. The echogenicity was increased, and the parenchymal structure was homogeneous. In the lower pole of the middle third of the spleen, a lesion measuring 1.8×1.6 cm with a heterogeneous internal structure and poorly defined borders was visualised, with no increase in blood flow to the lesion (Fig. 4). A laparotomic splenectomy was performed.

Macroscopic changes: Palpation of the spleen was not compressed, blood-filled, with a soft-consistency neoplasm located superficially (Fig. 5). **Morphological changes:** Histologically, the spleen showed age-related involutive changes, such as widespread hyalinisation of the capsule and sclerosis of the organ's parenchyma. There were areas of proliferating thick-walled fibrous structures branching and anastomosing to each other. These septa were lined with endothelial cells without signs of cellular atypia. The formations corresponded to cavernous structures, with the lumens partially filled with blood (Figs. 6, 7). The histological findings are consistent with a cavernous haemangioma.

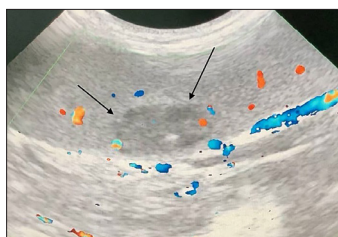


Figure 4. Ultrasound image of the splenic neoplasm

Note: neoplasm 1.8×1.6 cm

Source: authors' material

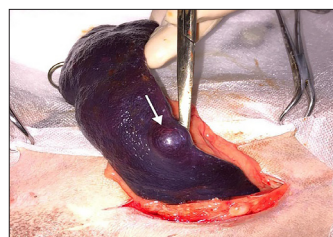


Figure 5. Intraoperative photo of the same splenic neoplasm

Note: neoplasm visualisation

Source: authors' material

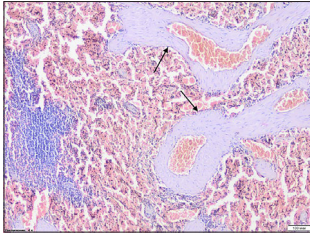


Figure 6. Proliferation of thick-walled septa lined with endothelial cells, forming cavernous structures

Note: Haematoxylin-eosin staining, magnification $\times 40$

Source: authors' material

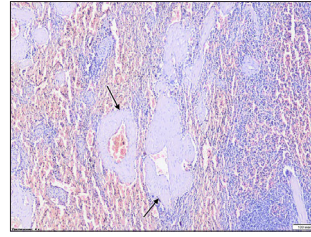


Figure 7. Proliferation of thick-walled septa lined with endothelial cells, forming cavernous structures

Note: Haematoxylin-eosin staining, magnification $\times 40$

Source: authors' material

Neoplasm complicated by haemorrhage.

Dog, Jack Russell Terrier, 11-years-old, female.

Medical history: The splenic neoplasm was detected during a dispensary examination of the animal via ultrasound examination. No clinical symptoms of the disease were recorded. **Ultrasound examination:** The spleen was neither enlarged nor displaced, maintaining its typical shape, with smooth contours and well-defined borders. The parenchymal structure was homogeneous, and coarse-grained, with unchanged echogenicity. In the mid-third of the spleen, a formation was found in the central pole, with clear contours measuring 1.5×1.4 cm, with a

hypoechoic internal structure (Fig. 8). A laparotomic splenectomy was performed. Macroscopically, the spleen was not enlarged, palpably compacted, blood-filled, and a focal point with structural disorders was found in the parenchyma. On incision, this area revealed a disruption in tissue integrity due to the accumulation of unstructured, dark red masses (Fig. 9). **Morphological changes:** Histologically, fragments of splenic parenchyma showed areas of haemorrhage replaced by erythrocytes, with a focus of haemorrhage in the white pulp, and areas of capsular hyalinisation (Fig. 10). The histological presentation is consistent with haemorrhage.

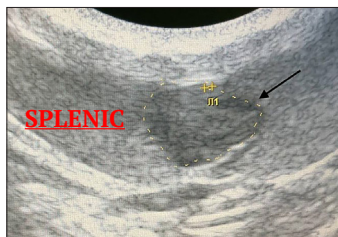


Figure 8. Ultrasound image of the splenic neoplasm

Note: neoplasm with mixed echogenicity 1.50×1.4 cm

Source: authors' material



Figure 9. Intraoperative photo of the same splenic neoplasm

Note: visualisation of haemorrhage within the parenchyma

Source: authors' material

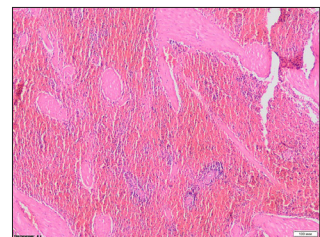


Figure 10. Spleen showing disrupted typical histostructure due to haemorrhage

Note: Haematoxylin-eosin staining, magnification $\times 40$

Source: authors' material

Lymphangiohaemangioma. Dog, French Bulldog, 11-years-old, female.

Medical history: The animal underwent surgery for purulent endometritis three months

prior. The splenic neoplasm was detected during a dispensary examination. Ultrasound examination: The spleen was not enlarged, its shape was typical, with smooth contours and well-defined borders. The parenchyma appeared homogenous, and echogenicity was unchanged. In the lower pole, an oval-shaped neoplasm measuring 2.2×1.5 cm was detected, with indistinct borders and a hypoechoic internal structure. A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, with a doughy consistency

on palpation and filled with blood. In the lower pole, a mass measuring 2.2×1.5 cm with a firm consistency was observed (Fig. 11). Morphological changes: Histologically, a tumour was found in the splenic parenchyma, consisting of proliferating thick- and thin-walled vessels with branching, narrow lumens. Some vascular spaces showed occlusion, while the majority were filled with lymph and blood. The endothelium exhibited signs of hyperchromasia and oedema (Figs. 12, 13). The histological findings are consistent with lymphangiohaemangioma.



Figure 11. Intraoperative photo of the splenic neoplasm

Note: neoplasm localisation
Source: authors' material

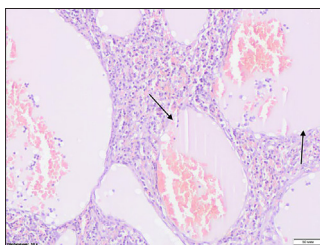


Figure 12. Splenic tissue with the proliferation of lymphangiohaemangioma, filled with lymph and blood

Note: Haematoxylin-eosin staining, magnification ×100
Source: authors' material

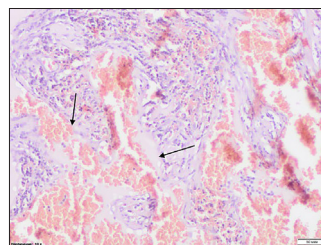


Figure 13. Lymphangiohaemangioma proliferation

Note: Haematoxylin-eosin staining, magnification ×100
Source: authors' material

Vascular malformation and haemorrhage. Dog, Yorkshire Terrier, 10-years-old, male.

Medical history: Clinical symptoms included abdominal discomfort, abdominal wall tremors, and a reaction to bimanual palpation. Ultrasound examination: The spleen was not enlarged, with a typical shape, smooth contours, and well-defined boundaries. Echogenicity was mixed, and the parenchymal structure appeared heterogeneous (Fig. 14). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was palpably compacted

with a focal structural disorder in the form of grey-brown foci with relatively clear contours (Fig. 15). Morphological changes: Histological examination of the splenic tissue revealed vascular malformation characterised by thick-walled vessels, branching and anastomosing with each other, alongside fields of haemorrhage with varying degrees of organisation. The white pulp was almost entirely displaced, with no signs of cellular atypia (Fig. 16). The histological findings indicate vascular malformation and haemorrhage.

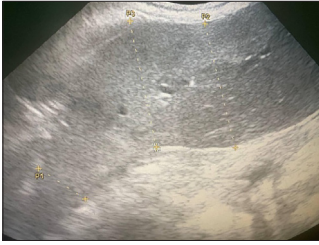


Figure 14. Ultrasound image of the splenic

Note: mixed echogenicity
Source: authors' material

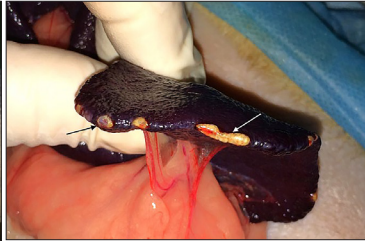


Figure 15. Intraoperative photo | of the same splenic

Note: isualisation of spleen tissue
Source: authors' material

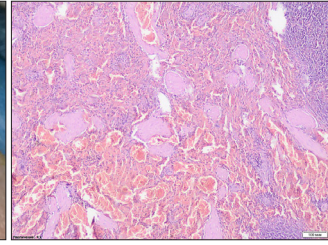


Figure 16. Spleen specimen demonstrating a focus of vascular malformation

Note: Haematoxylin-eosin staining, magnification $\times 40$
Source: authors' material

Old haemorrhage. Dog, Jack Russell Terrier, 10-years-old, male.

Medical history: A splenic neoplasm was discovered incidentally during the animal's routine check-up. No clinical symptoms of the disease were recorded. An ultrasound examination of the abdominal organs revealed that the spleen was not enlarged or displaced, but a localised area with clear contours and altered echostructure was found within the parenchyma (Fig. 17). A laparotomy was performed for splenectomy. A laparotomic splenectomy was performed.

Macroscopic changes: The spleen was not enlarged, had a doughy consistency upon palpation, and was filled with blood. An area measuring 2.5×2.3 cm was present in the middle third of the organ (Fig. 18). Morphological changes: Histologically, the typical architecture was disrupted due to the formation of a cavity, the walls of which comprised the parenchyma of the spleen, with areas of mildly expressed fibrosis. The cavity was filled with erythrocytes exhibiting signs of decay (Figs. 19, 20). The histological findings are characteristic of old haemorrhage.

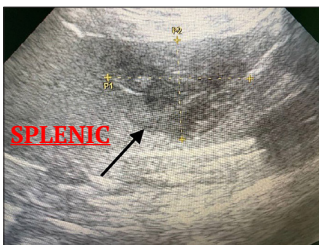


Figure 17. Ultrasound image of the splenic neoplasm

Note: neoplasm with mixed echogenicity 2.50×2.3 cm
Source: authors' material



Figure 18. Intraoperative photo of the same splenic neoplasm

Note: visualisation of haemorrhage
Source: authors' material

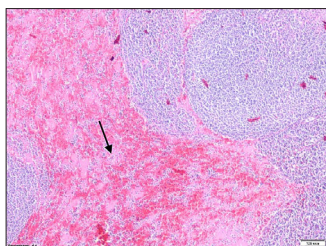


Figure 19. Spleen showing disrupted typical histostructure due to haemorrhage

Note: Haematoxylin-eosin staining, magnification $\times 100$

Source: authors' material

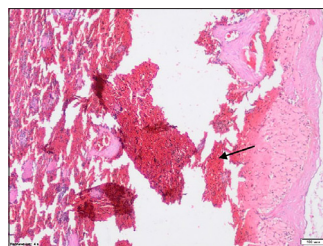


Figure 20. Subcapsular area of the spleen with disrupted typical histostructure due to haemorrhage

Note: Haematoxylin-eosin staining, magnification $\times 100$

Source: authors' material

Splenic lymphoma. Dog, mixed breed, 10-years-old, male.

Medical history: The animal exhibited weight loss and intermittent vomiting, particularly in the morning on an empty stomach. During a clinical examination using ultrasound of the abdominal organs, echostructural signs of a neoplasm were observed in the parenchyma of the spleen. The spleen was not enlarged, retained its typical shape, had smooth contours,

and clear margins. Echogenicity was increased, and the parenchymal structure was homogeneous. A formation with increased echogenicity measuring 1.8×1.6 cm was visualised in the upper pole of the parenchyma (Fig. 21). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was palpably compressed and blood-filled, with a dense formation in the upper pole of the parenchyma measuring 1.8×1.6 cm (Fig. 22).

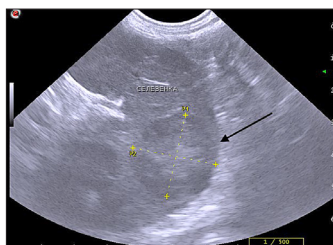


Figure 21. Ultrasound image of a splenic neoplasm (local changes in echostructure)

Note: neoplasm of the spleen with increased echogenicity

Source: authors' material

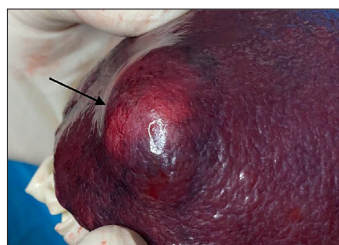


Figure 22. Intraoperative photo of the same neoplasm in the spleen

Note: localisation of lymphoma

Source: authors' material

Morphological changes: The histological structure of the spleen's parenchyma was completely disrupted due to the proliferation of neoplastic lymphoid-type cells, resulting in a diffuse growth pattern. The tumour cells were medium-sized, possessing a single enlarged

nucleus with convoluted and diffuse chromatin, and exhibited figures of mitotic division. The cytoplasm of the cells appeared limited, amphiphilic, or weakly basophilic (Figs. 23, 24). The histological findings are consistent with lymphoma.

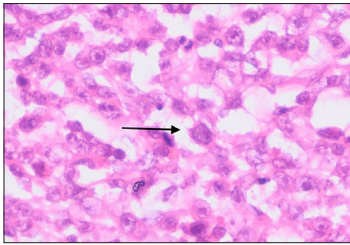


Figure 23. Spleen exhibiting disrupted typical histostructure due to the proliferation of pleomorphic neoplastic cells of lymphoid origin

Note: Haematoxylin-eosin staining, magnification $\times 200$
Source: authors' material

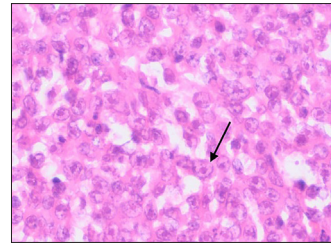


Figure 24. Spleen exhibiting disrupted typical histostructure due to the proliferation of pleomorphic neoplastic cells of lymphoid origin

Note: Haematoxylin-eosin staining, magnification $\times 100$
Source: authors' material

Haemangiolymphangioma. Dog, Yorkshire Terrier, 10-years-old, female.

Medical history: No clinical symptoms of the disease were recorded. **Ultrasound examination:** The spleen was not enlarged, with a typical shape, smooth contours, and clear margins. Echogenicity was elevated, and the parenchyma exhibited a homogeneous structure. In the middle third of the organ, a formation measuring 1.1×1.0 cm was visualised, characterised by indistinct contours and a hypoechoic internal structure (Fig. 25).

A laparotomic splenectomy was performed. **Macroscopic changes:** The spleen was not

enlarged, and the parenchyma was full of blood, with a moderately dense neoplasm palpable beneath the capsule (Fig. 26). **Morphological changes:** Histologically, the neoplasm was represented by a system of vascular structures separated by thin connective tissue septa. The lumens of the vessels varied in size, and the walls were lined with endothelial-type epithelial cells. The lumens of the vascular structures were partially filled with homogeneous eosinophilic material corresponding to lymph, and partially with erythrocytes (Figs. 27, 28). The histological picture is characteristic of haemangiolymphangioma.

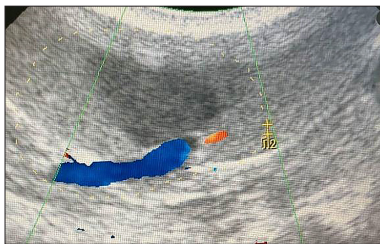


Figure 25. Ultrasound and Doppler examination of the spleen

Note: area with a hypoechoic internal structure
Source: authors' material



Figure 26. Intraoperative photo of the neoplasm in the spleen

Note: haemangiolymphangioma
Source: authors' material

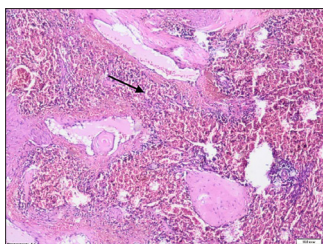


Figure 27. Tumour composed of lymphatic and blood vessel types

Note: Haematoxylin-eosin staining, magnification $\times 40$

Source: authors' material

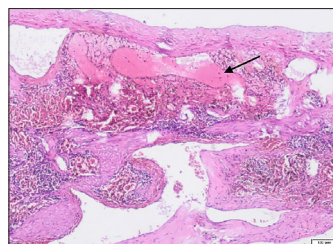


Figure 28. Structures of the haemangiolympangioma that branch and anastomose with one another

Note: Haematoxylin-eosin staining, magnification $\times 40$

Source: authors' material

Cavernous lymphangioma of the spleen.

Dog, Pug, 9-year-old, female.

Medical history: Clinical symptoms of the disease were not observed. During a screening examination using ultrasound of the abdominal organs, a neoplasm was identified in the parenchyma of the spleen. A laparotomic splenectomy was performed. Macroscopic changes: The spleen was enlarged and engorged with blood, a compacted area

with a hard consistency was palpated in the parenchyma (Fig. 29). Morphological changes: Histologically, the lumens of the vascular structures were filled with a homogeneous light eosinophilic substance corresponding to lymph, within which a few lymphoid cells were diffusely distributed, showing no signs of cellular atypia (Figs. 30, 31). The histological picture is consistent with cavernous lymphangioma.

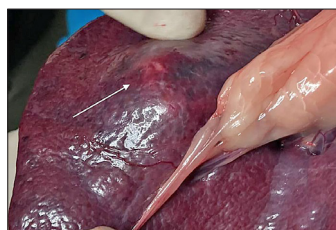


Figure 29. Intraoperative photo of the cavernous lymphangioma

Note: area with parenchymal damage

Source: authors' material

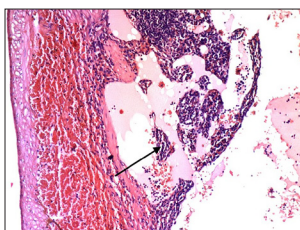


Figure 30. Cavernous lymphangioma of the spleen

Note: Haematoxylin-eosin staining, magnification $\times 100$

Source: authors' material

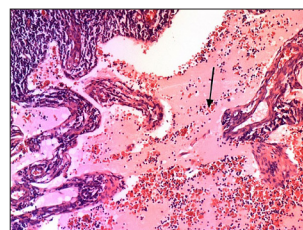


Figure 31. Cavernous lymphangioma of the spleen

Note: Haematoxylin-eosin staining, magnification $\times 100$

Source: authors' material

Cavernous haemangioma. Dog, Yorkshire Terrier, 8-years-old, male.

Medical history: The animal exhibited no clinical signs of illness. A neoplasm in the parenchyma of the spleen was discovered

incidentally during an ultrasound examination of the abdominal organs. Ultrasound examination: The spleen was not enlarged, and had a typical shape, with smooth contours and clear boundaries. Echogenicity was elevated, and

the structure of the parenchyma was coarse-grained and homogeneous. A formation measuring 1.02×1.3 cm was identified, with indistinct contours and an internal structure of mixed echogenicity (Fig. 32). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, engorged with blood, and palpably not compacted. Under the capsule, a neoplasm was visualised that differed in colour from the parenchyma, exhibiting a soft consistency (Fig. 33). Morphological



Figure 32. Echostructural changes in the parenchyma with a neoplasm of mixed echogenicity

Note: neoplasm 1.02×1.30 cm

Source: authors' material

changes: Histologically, a tumorous formation was identified, comprising thick-walled vessels with cavernous structures. The inner walls of the cavities were lined with endothelial cells, the vessel is surrounded by serocytes, without histological features. The cavities of the formation were filled with erythrocytes exhibiting signs of decay, along with the formation of mural thrombi (Figs. 34, 35). The histological picture supports a diagnosis of cavernous haemangioma.



Figure 33. Intraoperative material.

Visual changes in the spleen

Note: localised cavernous haemangioma

Source: authors' material

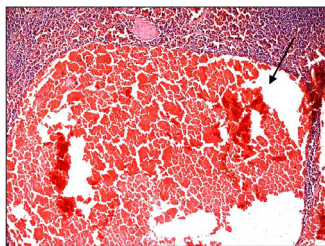


Figure 34. Cavernous haemangioma composed of dilated vessels forming cavities filled with blood

Note: Haematoxylin-eosin staining, magnification ×100

Source: authors' material

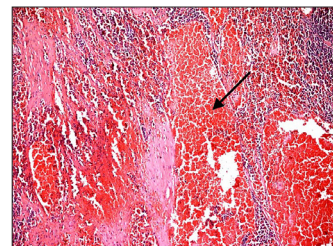


Figure 35. Cavernous haemangioma composed of dilated vessels forming cavities filled with blood

Note: Haematoxylin-eosin staining, magnification ×100

Source: authors' material

Lymphogranulomatosis (Hodgkin's lymphoma). Cheetah (*Acinonyx jubatus*), name: Casper, 3-years-old, male. Medical history: The animal exhibited a decrease in appetite, gradual weight loss, and digestive disturbances. During an abdominal ultrasound examination,

diffuse neoplasms of varying sizes and mixed echogenicity were identified within the splenic parenchyma (Fig. 36). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, exhibited insufficient blood supply, and had a doughy consistency.

Diffuse subcapsular neoplasms were present, which felt soft upon palpation (Fig. 37). Morphological changes: Histologically, there was the proliferation of lymphoid elements of varying degrees of maturity within the splenic tissue. Reed-Sternberg multinucleated cells and



Figure 36. Ultrasound image of the diffuse lesion in the splenic parenchyma

Note: mixed echogenicity of the parenchyma

Source: authors' material

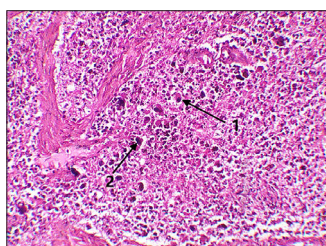


Figure 38. Lymphogranulomatosis (Hodgkin's lymphoma)

Note: a significant number of diagnostic cells: 1 – Hodgkin's cells and 2 – Reed-Sternberg cells. Haematoxylin-eosin staining, magnification $\times 200$

Source: authors' material

Myelolipoma. Cheetah (*Acinonyx jubatus*), name: Orion, 6-years-old, male.

Medical history: The animal exhibited limited physical activity, an increase in the contour of the abdominal wall, and dyspeptic disorders. Ultrasound examination revealed diffuse changes in the echostructure of the spleen, characterised by mixed echogenicity. A laparotomic splenectomy was performed. Macroscopic changes: The spleen was enlarged and firm, with diffuse neoplastic formations of

Hodgkin's single-nucleus cells were identified. The background was comprised of an infiltrate of lymphocytes and eosinophils. Tumour structures were partially separated by connective tissue strands (Figs. 38, 39). The histological appearance is characteristic of lymphogranulomatosis.



Figure 37. Intraoperative photo.

Macroscopic changes

Note: diffuse lesions in the splenic parenchyma

Source: authors' material

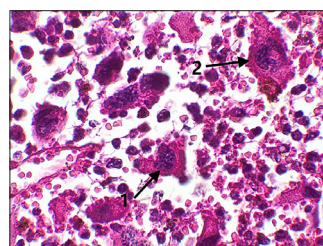


Figure 39. Lymphogranulomatosis (Hodgkin's lymphoma)

Note: a significant number of diagnostic cells: 1 – Hodgkin's cells and 2 – Reed-Sternberg cells. Haematoxylin-eosin staining, magnification $\times 1000$

Source: authors' material

solid consistency noted within the parenchyma (Fig. 40). Morphological changes: Histologically, the tumour tissue was composed of mature lipocytes with large, optically clear cytoplasm and a single eccentrically located nucleus, showing no signs of nuclear pleomorphism. Deposits of myeloid-derived cells were observed among the proliferates of mature lipocytes, with no evidence of cellular atypia (Figs. 41, 42). The histological appearance is characteristic of myelolipoma.



Figure 40. Intraoperative photo of the spleen
Note: diffuse splenic lesions
Source: authors' material

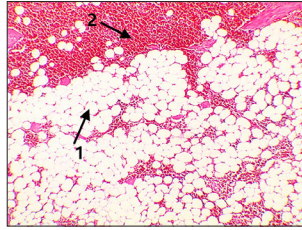


Figure 41. Myelolipoma. Proliferation of lipocytes and myeloid cells
Note: 1 – lipocytes; 2 – myeloid cells. Haematoxylin-eosin staining, magnification $\times 100$
Source: authors' material

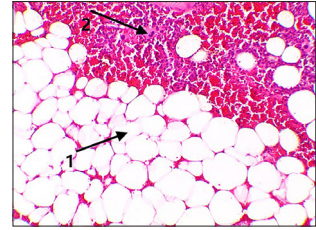


Figure 42. Myelolipoma. Proliferation of lipocytes and myeloid cells
Note: 1 – lipocytes; 2 – myeloid cells. Haematoxylin-eosin staining, magnification $\times 200$
Source: authors' material

Myelolipoma. Cheetah (*Acinonyx jubatus*), name: Ariel, 6-years-old, female. Medical history: No clinical symptoms of the disease were reported. The examination was carried out in a dispensary using ultrasound. Splenomegaly and solitary hyperechoic inclusions were detected in the splenic parenchyma (Fig. 43). A laparotomic splenectomy was performed. Macroscopic changes in the organ: The spleen was engorged with blood, enlarged, and had a doughy consistency upon palpation.

Various-sized compacted inclusions were noted beneath the capsule (Fig. 44). Morphological changes: Histologically, the tumour tissue consisted of mature lipocytes with voluminous optically clear cytoplasm and a single eccentrically located nucleus, with no signs of nuclear pleomorphism. Between the proliferation of mature lipocytes, deposits of myeloid cells were observed, with no evidence of cellular atypia (Figs. 45, 46). The histological picture is consistent with myelolipoma.

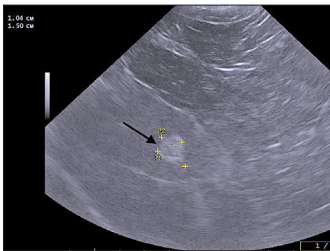


Figure 43. Ultrasound image of the neoplasm
Note: area with a hyperechoic lesion measuring 1.04x1.5 cm
Source: authors' material



Figure 44. Intraoperative photo of the spleen
Note: hyperaemia, splenomegaly, and solitary neoplasms in the spleen
Source: authors' material

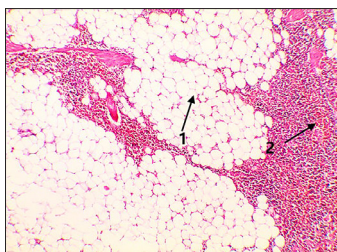


Figure 45. Lipoma (myelolipoma).

Proliferation of lipocytes and myeloid cells

Note: 1 – lipocytes; 2 – myeloid cells. Haematoxylin-eosin staining, magnification $\times 100$

Source: authors' material

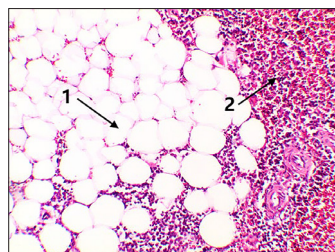


Figure 46. Lipoma (myelolipoma).

Proliferation of lipocytes and myeloid cells

Note: 1 – lipocytes; 2 – myeloid cells. Haematoxylin-eosin staining, magnification $\times 100$

Source: authors' material

Thus, the described results of ultrasound diagnostics, surgical interventions (splenectomy), and histopathological studies of the spleen are consistent with the findings of C.H. Spröhnle-Barrera *et al.* (2022), which state that the spleen can be affected by various tumours, such as tumour-like formations, vascular tumours, lymphoid tumours, and non-lymphoid tumours. Establishing a clinical diagnosis during the animal's lifetime remains challenging when dealing with pathological changes directly within the organ. Ultrasound diagnostics clearly revealed pathological structural changes, their size, quantity, and whether the organ was enlarged or reduced (hypersplenism or hyposplenism); however, the diagnosis is confirmed through morphological examination.

A. Auerbach *et al.* (2023) highlighted the challenges of differential diagnosis of splenic neoplasms, particularly regarding surgical biopsy methods and the complications that may arise following the procedure. The authors emphasised the importance of developing molecular-genetic markers for tumour identification. In their studies, patients in the postoperative period were provided with adequate antibacterial therapy and postoperative pain management. Special attention was given to wild animals, particularly cheetahs (*Acinonyx jubatus*), as postoperative access to these animals was

limited (making it difficult to maintain surgical sutures). In the operated animals, the surgical wound healed by primary intention (*prima intentio*) on the 10th-12th day, without signs of complications.

A.M. Pîrvu *et al.* (2021) demonstrated the diversity of morphological changes using 194 cases of surgically removed spleens. The authors characterised the macroscopic appearance of the spleen in cases of splenomegaly, cystic, and tumorous pathological lesions. The study focused on postoperative cytological and histopathological modifications. In cases of splenomegaly, the absence of follicles and dendritic cells, as well as poorly developed fibrous trabeculae, were noteworthy. Additionally, foci of extramedullary haematopoiesis may be observed. In cavernous haemangiomas, the tumour was composed of vascular cavities resembling sinusoids of varying sizes and shapes, interconnected with one another. These cavities were lined by a single layer of compressed endothelial cells. Similar findings were obtained in this study. The authors also emphasised that such haemangiomas can lead to spontaneous splenic rupture and may give rise to the following vascular tumours: cavernous haemangioma, littoral cell angioma, multinodular haemangioma, capillary haemangioma, benign (infantile) haemangioendothelioma, diffuse

sinusoidal haemangiomatosis, angiosarcoma, and haemangiopericytoma.

According to Y.U. Ko *et al.* (2023), histopathological studies are crucial for predicting the course of angiosarcoma. Angiosarcoma was found to be the most common malignant vascular tumour of the spleen. Its microstructure is polymorphic, characterised by spindle-shaped, polygonal, or round cells forming vascular channels and slits. In these studies, angiosarcoma appeared macroscopically as a distinct haemorrhagic nodule, with a risk of organ rupture. Microscopically, large vascular cavities filled with blood were observed, lined by a thin layer of immature endothelial cells (tumoural angioblasts). Focal areas of loose connective tissue fibres were found between tumour cells. The neoplastic endothelium was loose, leading to frequent signs of wall integrity disruption within the tumoural vessels, which resulted in haemorrhages and necrosis. The nuclei of tumoural angioblasts were enlarged, polymorphic, and hyperchromatic.

One type of malignant transformation of lymphoid tissue is lymphogranulomatosis, or Hodgkin's lymphoma, characterised by the presence of Reed-Sternberg giant cells, which are identified during microscopic examination of affected lymph nodes. In this study, numerous Reed-Sternberg cell proliferations were found not only in the lymph nodes but also in the spleen, indicating an aggressive malignant progression and extensive spread of the disease.

Y.U. Ko *et al.* (2023) demonstrated that myelolipoma belongs to the category of non-lymphoid tumours and is significantly less common compared to other spleen neoplasms. This polyetiological disease is based on various toxic injuries to the spleen, such as infections, lymphatic system tumours, autoimmune and systemic diseases, haemolytic anaemias, and others. It leads to splenomegaly and replacement of the parenchyma with adipose and

myeloid tissue. This condition poses a threat as it alters the organ's topography and structure, potentially leading to torsion or even rupture. In the research described in this study, myelolipoma was recorded in two cheetahs (*Acinonyx jubatus*) and confirmed morphologically. In one case, clinical symptoms characteristic of spleen neoplasms were observed, while the second case was subclinical. After surgical intervention (splenectomy), the symptoms in the affected animal disappeared.

Clinical and morphological studies of splenic lesions confirm that neoplasms can be of various types. Early detection, surgical intervention, and histological examination are crucial for the detailed characterisation of the pathological condition and saving the animal's life.

Conclusions

When neoplasms develop in the spleen of animals, it becomes necessary to verify the structural changes to refine the clinical diagnosis and, consequently, the prognosis. Currently, the primary diagnostic method for splenic neoplasms (such as cysts and tumours) in veterinary medicine is abdominal ultrasound, as it is the most accessible for patients. However, based on experience and the capabilities of ultrasound equipment, the nature of the changes can be inferred from the echographic image of the organ's structure. In human medicine, diagnostic computed tomography and magnetic resonance imaging are also widely used.

All methods of instrumental visual diagnostics were aimed at a more detailed study of the pathomorphological changes in the spleen and the development of individualised therapeutic strategies or surgical interventions. The conducted studies show that neoplastic processes in the spleen parenchyma appeared in most cases in adult animals, predominantly in the second half of life. In the case of diagnosed benign neoplasms, the possibility of

threatening conditions cannot be excluded, namely: rupture of the spleen capsule and leakage of the contained neoplasm into the abdominal cavity, which may lead to the development of haemoperitoneum or peritonitis; or the abscessing of the neoplasm if a bacterial infection occurs.

In cases where a malignant tumour or even signs of its presence are established, splenectomy is warranted. All detected neoplasms in the splenic parenchyma should undergo further morphological examination following the splenectomy. Post-splenectomy morphological studies provide the opportunity to establish a definitive clinical diagnosis and to predict the course of the disease, enabling the development of an individualised therapeutic strategy for patient management.

According to the conducted research, splenic neoplasms are often interpreted as “incidental findings” in dispensary ultrasound screening. In such cases, disease symptoms

are absent. Timely surgical intervention and subsequent morphological examination can objectively contribute to extending the animal's life when benign neoplasms are present (such as haemangiolymphangioma, lymphangiohaemangioma, cavernous haemangioma, cavernous lymphangioma, haemorrhage, and myelolipoma). In cases of malignant neoplasms (lymphoma, lymphogranulomatosis, non-Hodgkin lymphoma), it has also been possible to manage the disease effectively and alleviate the recovery period.

In the future, it is planned to implement the developed experimental immunohistochemical techniques and oncological markers.

Acknowledgements

None.

Conflict of Interest

The authors declare no conflict of interest.

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Анотація. Актуальність дослідження на сьогодні зумовлена гострою необхідністю у поглибленому вивченні морфологічних аспектів ідентифікації вогнищевих і осередкових уражень в селезінці. Остання здійснює низку життєво необхідних функцій, а саме: імунну, гематологічну, депонуючу, обмінну, гемостатичну, гормональну, антибластичну тощо. Тому,

мета цього дослідження стосувалася визначення особливостей клінічного прояву пухлинного ураження селезінки та її структурних змін, а також визначення макроскопічної картини органу після спленектомії. У діагностиці захворювань селезінки використовували провідний інструментальний метод, такий як ультразвукове дослідження з доплерографією, що дозволило комплексно дослідити, проаналізувати та охарактеризувати патології в паренхімі органу. Тваринам, під час диспансеризації або за ознак захворювання проводили клінічне обстеження. Після виявлення новоутворення селезінки здійснювали хірургічне лікування, а саме, оперативне втручання методом відкритої лапаротомії та спленектомію. Операція проводилася під комбінованим загальним знеболюванням (неінгаляційні та інгаляційні анестетики). Відібраний інтраопераційно матеріал із ураженого органу підлягав гістологічному дослідженню. З'ясували, що патоморфоз уражень селезінки різний, а власні патологістологічні дослідження висвітлюють основні морфологічні особливості новоутворень. Відповідно, будь-яке новоутворення селезінки може спричинити загрозливий стан з багатьох причин: розрив органу та кровотечу, скручування органу, малігнізацію тощо. Отримані результати мають вирішальне значення у верифікації патології селезінки, що дозволяє поставити заключний клінічний діагноз, провести лікування тварини та подовжити тривалість її життя. Практичне значення отриманих результатів полягає у виявленні необхідності комплексного проведення клінічних і морфологічних досліджень у діагностиці захворювань селезінки, а також дослідженні гістопрепаратів на предмет клітинних та тканинних змін. Дискусійним у ветеринарній медицині може бути метод хірургічного лікування в тварин уражень селезінки. На противагу, в гуманній медицині вже визначені алгоритми проведення органоруйнуючої операції, – спленектомії (відкритим або лапароскопічним доступом), органозберігаючої – резекція селезінки або спленектомія з аутотрансплантацією селезінкової тканини у великий сальник (відкритим або лапароскопічним доступом), через шкірні пункції при кістозних утвореннях селезінки

Ключові слова: ультразвукове дослідження; лімфогранулематоз; мієлоліпома; структурні зміни; лапаротомія; ендотеліальні клітини; неходжкінська лімфома