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CANINE SPLENIC HEMANGIOSARCOMA: LITERATURE REVIEW AND EXPERIENCE AT THE VETERINARY TEACHING HOSPITAL OF THE UNIVERSITY OF PARMA

EMANGIOSARCOMA SPLENICO CANINO: *REVIEW* DELLA LETTERATURA ED ESPERIENZA PRESSO L'OSPEDALE VETERINARIO UNIVERSITARIO DIDATTICO DELL'UNIVERSITA' DI PARMA

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SUMMARY

1. ABSTRACT

The spleen is an organ commonly affected by benign and malignant, primary and metastatic neoplasms. Hemangiosarcoma is the most common splenic malignant tumor found in dogs; in particular, it represents 44-65% of malignant splenic neoformations (O'Byrne & Hosgood, 2019; Sherwood, et al., 2016; Day, et al., 1995; Johnson, et al., 1989).

The aim of this retrospective study is to analyze patients' data with spleen neoformations undergoing surgery in order to evaluate their survival times, compare them with the literature and highlight any prognostic factors.

54 dogs were included in the University Veterinary Hospital of the University of Parma in a period between 1st January 2014 and 1st April 2022. All patients underwent abdominal ultrasound and chest X-ray, except for 3 dogs, which have undergone CT; 5 dogs performed liver and spleen FNA. The most represented breed is given by mixed-breed, followed by German Shepherd, Boxer and Labrador Retriever dogs. 23 whole females, 8 spayed females, 22 whole males and only one neutered dog were subjected to splenectomy, while 60% of the splenectomized dogs with splenic hemangiosarcoma were female, although there is no statistically significant difference between the two genders in terms of survival. The median age of onset was 10 years and 9 years for splenic hemangiosarcoma. Histologically, 68.5% of the dogs had a benign tumor while 31.5% had a malignant tumor; among these, nearly 60% of dogs presented with splenic hemangiosarcoma; this is in contrast with the so-called "*2/3 rule*" reported in the literature, according to which 2/3 of splenic neoformations are malignant and 2/3 of these are hemangiosarcomas (Steve, et al., 2016; Davies & Taylor, 2020). Furthermore, 53.5% of the dogs presented concomitant pathologies at the time of surgery and almost 10% were affected by other neoplasms, discovered after surgery; statistically, these comorbidities did not affect survival times. The splenic neoformations had a diameter ranging from 1 to 30 cm; in particular, the splenic hemangiosarcomas had a diameter of less than 10 cm. As for the *MST*, about 50% of subjects undergoing splenectomy for any type of splenic neoformation survive for more than one year while the *MST* of dogs with splenic hemangiosarcoma lasted 124.5 days (109 days excluding two outliers). About any prognostic factors such as individual characteristics, comorbidities or the presence of hemoabdomen, found in 90% of dogs with hemangiosarcoma, it is evident that only the abdominal bleeding represents a negative prognostic factor.

ABSTRACT

La milza è un organo comunemente interessato da neoplasie benigne e maligne, primitive e metastatiche. L'emangiosarcoma è il tumore maligno splenico maggiormente riscontrato nei cani; in particolare, rappresenta il 44-65% delle neoformazioni spleniche maligne (O'Byrne & Hosgood, 2019; Sherwood, et al., 2016; Day, et al., 1995; Johnson, et al., 1989).

L'obiettivo del presente studio retrospettivo consiste nell'analizzare i dati dei pazienti presentanti neoformazioni di pertinenza splenica e sottoposti a chirurgia al fine di valutarne i tempi di sopravvivenza, confrontarli con i dati riportati in letteratura ed evidenziare eventuali fattori prognostici.

Sono stati inclusi 54 cani afferiti all'Ospedale Veterinario Didattico Universitario dell'Università di Parma in un periodo compreso tra il 1° Gennaio 2014 e 1° Aprile 2022. Tutti i pazienti sono stati sottoposti ad ecografia addominale e radiografia toracica, ad eccezione di 3 cani, i quali sono stati sottoposti a TC; 5 cani hanno eseguito FNA di fegato e milza. La razza maggiormente rappresentata è data dai meticci, seguiti da cani di razza Pastore Tedesco, Boxer e Labrador Retriever. Sono stati sottoposti a splenectomia 23 femmine intere, 8 femmine sterilizzate, 22 maschi interi e un maschio castrato. Il 60% degli animali splenectomizzati con emangiosarcoma splenico era di sesso femminile, anche se non esiste una differenza statisticamente significativa tra i due generi in termini di sopravvivenza. L'età di insorgenza mediana era di 10 anni e in particolare di 9 anni per l'emangiosarcoma splenico. Istologicamente, il 68.5% dei cani era affetto da un tumore di natura benigna mentre il rimanente 31.5% presentava un tumore maligno; tra quest'ultimi, il 58.8% dei cani presentava emangiosarcoma splenico; ciò è in contrapposizione con la cosiddetta "*regola dei 2/3*" riportata in letteratura, secondo cui 2/3 delle neoformazioni spleniche sono maligne e i 2/3 di questi sono emangiosarcomi (Steve, et al., 2016; Davies & Taylor, 2020). Inoltre, il 53.5% dei cani presentava patologie concomitanti al momento della chirurgia e quasi il 10% era affetto da altre neoplasie, rinvenute in tempi successivi alla chirurgia; dal punto di vista statistico, queste comorbidità non hanno influenzato i tempi di sopravvivenza. Le neoformazioni spleniche avevano un diametro variabile da 1 a 30 cm; in particolare, gli emangiosarcomi splenici presentavano un diametro inferiore ai 10 cm. Per quanto riguarda il *MST*, si evidenzia che circa il 50% dei soggetti sottoposti a splenectomia per qualsiasi tipologia di neoformazione splenica sopravvive per più di un anno mentre il *MST* dei cani con emangiosarcoma splenico è stato di 124.5 giorni (109 giorni escludendo due outliers). Circa eventuali fattori prognostici quali caratteristiche individuali, comorbidità o la presenza di emoaddome, rinvenibile

nel 90% dei cani con emangiosarcoma, si evince che solo l'emoaddome rappresenta un fattore prognostico negativo.

2. INTRODUCTION

2.1 SPLEEN ANATOMY

The spleen is an unequal organ that is closely attached to the stomach by the gastrosplenic ligament and is situated in the abdominal cavity at the level of the 11^{th} to 12^{th} intercostal gap, in touch with the abdominal wall, depending on the degree of repletion of the stomach. The ventral extremity of the spleen is situated caudal to the left costal arch, but it can also move to the right side of the body, behind the midline, in cases of incomplete stomach repletion (Budras, et al., 2011). It has two extremities, two dorsal and visceral faces and two cranial and caudal margins.

One or more smaller accessory spleens may occasionally be found in the gastro-lienal ligament or close to the hilum. Each of these flattened or spheroidal structures receives a peduncle of the lienal vessels (Barone, 2014). The gastro-lienal ligament, which connects the spleen to the great gastric curvature, extends from the gastro-phrenic ligament to the left diaphragmatic pillar and ends at the left kidney. It then continues as the phrenic-lienal or lieno-renal ligament (Pelagalli & Botte, 2001).

The splenic parenchyma consists of red pulp and white pulp. The white pulp is made up of diffuse and nodular lymphoid tissue and is the site of the immunological response, whereas the red pulp is responsible for storing erythrocytes and trapping antigens (Spencer & Tobias, 2020).

2.2 SPLEEN VASCULARIZATION AND INNERVATION

The *splenic artery*, which arises from the celiac trunk (a branch of the abdominal aorta), supplies the spleen, crosses the gastric fundus and the left extremity of the pancreas before entering the gastro-lienal ligament and reaching the splenic hilum (Barone, 2014). After entering the spleen, *splenic arteries* immediately lose their vascular identity and enter the red pulp (Ettinger, et al., 2019). Basically, this organ is organized as a network of branched arterial blood vessels, with the smaller arterioles branching into the sinusoidal venous system (Mebius & Kraal, 2005).

The roots of the *splenic vein*, which is one of the roots of the portal vein, are formed by *trabecular veins* that are drained by satellite arteries (Pelagalli & Botte, 2001).

The *lymphatic vessels* create a deep and a superficial network that communicates with the lymph nodes along the hilum. The deep one is located in the trabeculae and directly reaches the ileum. The superficial one, however, has a vast number of vessels (Barone, 2014), (**Figure 1**).

The splenic artery is accompanied by *nerves* from the *celiac plexus*; this complex forms the extended *lienal plexus* (Barone, 2014). These nerves are directly descended from the vagus nerve and sympathetic nervous system (Pelagalli & Botte, 2001).

2.3 SPLEEN FUNCTIONS

The spleen has multiple functions, including (Poli, et al., 2017):

- remove microorganisms, cellular debris and aged cells from the bloodstream;
- act as a deposit of erythrocytes and platelets: in fact, it can store 10% to 20% of a dog's red blood cell mass and 30% of platelet mass (Madewell, 2000);
- regulate circulation in the portal vein area.

In fetus, it also has an important erythropoietic function, allowing the final maturation of red blood cells before being released into the systemic circulation.

Despite its different functions, the spleen is not necessary for animals to survive: in case of remotion, its function could be supplied by lymph nodes and bone marrow (Barone, 2014).

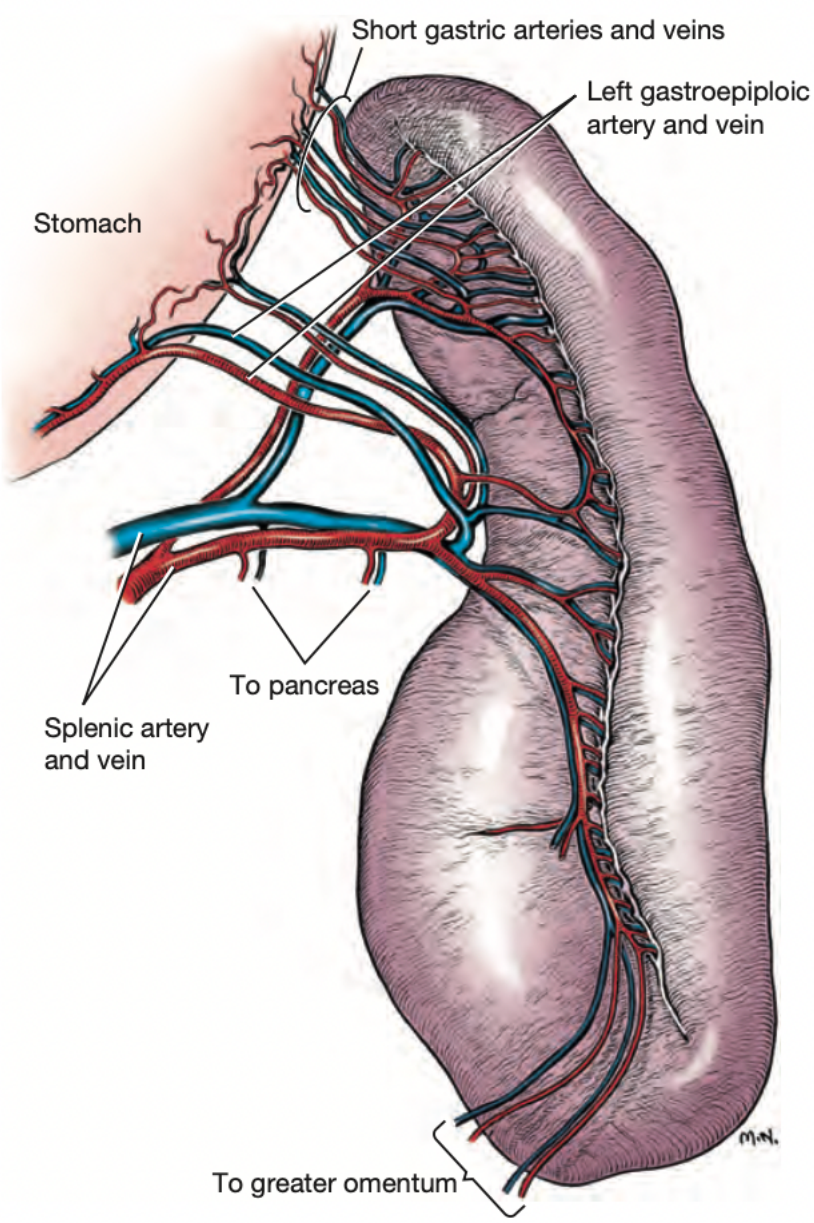

Figure 1 – *Splenic vascularization* (Evans & de Lahunta, 2012).

2.4 SPLENIC DISEASES

Many splenic diseases are sporadic findings, and dogs tend to exhibit nonspecific symptoms, such as (Mullin & Clifford, 2019):

- nausea
- vomiting
- weakness
- asthenia
- anorexia
- pale mucous membranes
- abdominal distension

2.4.1 CONGENITAL DISEASES

Spleen dislocation in thoracic cavity. Condition in which the spleen can be found in the cranial or caudal mediastinum, as well as inside the pericardium or pleural cavity, in addition to other organs (Marcato, 2015).

Accessory spleens. Presence of one or more patches of ectopic splenic tissue of varying sizes and shapes - circular or triangular - dispersed throughout the perisplenic region and vascularized by the same splenic artery branches. It is a common finding in dogs and cats. It can also result from self-implantation of splenic tissue after a splenectomy or trauma. Pancreas, gastrosplenic ligaments, and the splenic hilum are the most common implantation sites. This splenic tissue can be impacted by the same neoplasms and other pathological conditions that affect the spleen itself (Rossi, et al., 2010).

2.4.2 TRAUMA

Spleen torsion. Common pathology that might result from gastric dilatation and volvulus syndrome (*GDV*) or primarily affect the spleen. Due to the laxity of the gastrosplenic ligament, it is mainly observed in large-giant canine breeds. The etiology of primary splenic torsion is still unknown, and it may have both an acute and chronic course: the acute form results in circulatory collapse and shock-like symptoms, while the chronic type is challenging to diagnose due to diffuse clinical indications (Ohta, et al., 2009). Initially, it results in venous occlusion, which leads to splenic congestion, and only later it results in arterial congestion with splenic infarction (McGavin & Zachary, 2010).

Splenic rupture. Pathological phenomenon resulting from acute infectious splenomegaly, diffuse amyloidosis, neoformations and blunt (e.g. car accident or fall from a height) or penetrating trauma, such as from a knife or gunshot wound (DeHoff, et al., 1972). A small tear in the capsule can also act as the site of the rupture. If the rupture does not result in death, it may heal or create a hematoma; more frequently, it determines hemoperitoneum, which makes it a surgical emergency (Marcato, 2015).

2.4.3 CIRCULATION DISORDERS

Splenic anemia. It is often associated with atrophy in cachexia, or in association with stenosing arteriopathies. The spleen is smaller and paler, with a shriveled splenic capsule.

Splenic infarction. In dogs, it is a rare condition that accounts for 1 to 2% of splenic lesions (Hardie, et al., 1995). It is due to the occlusion of the splenic artery branches. There are two different forms: *ischemic*, which recognizes thromboendocarditis of the left heart as the main cause, and *hemorrhagic*, which has the appearance of an inverted wedge along the organ's margins (Marcato, 2015).

2.4.4 REGRESSIVE AND INFILTRATING PROCESSES

Atrophy. Regressive process resulting from stasis and chronic inflammation.

Amyloidosis. It is a frequent involutionary pathological phenomenon in older dogs, in which the amyloid substance tends to settle in the follicles and/or in the red pulp. Macroscopically, the splenic follicles appear as lardaceous and shiny nodules. When amyloidosis occurs only in the red pulp, there is a significant increase in the volume of the organ, accompanied by fragility of the entire splenic tissue.

Hemosiderosis. Phenomenon that determines a pathological increase in the deposit of hemosiderin in the red pulp, which takes on a characteristic rust color.

Necrosis. Regressive process resulting from local circulation disturbances or due to an infectious agent.

2.4.5 INFLAMMATION

Dogs rarely get splenitis, which are inflammatory disorders that damage the splenic parenchyma. Studies on this species have reported a prevalence ranging from 0.9 to 8% (Ferri, et al., 2017). It is usually associated with bacterial, viral, fungal or protozoal exposure (Robertson & Newman, 2000).

According to Ferri et al. (2017), the main bacteria found in dogs with splenitis are:

- *Staphylococcus spp.*
- *Mycobacterium avium Mycobacterium tuberculosis*
- *Listeria monocytogenes*
- *Bartonella henselae* and *vinsonii*
- *Clostridium spp.*
- *Bacillus anthacis*
- *Burkholderia pseudomallei*

In these cases, splenic lesions are pyogranulomatous or neutrophilic.

According to Ferri et al. (2017), the most frequently found protozoa are:

- *Neospora caninum*
- *Hepatozoon canis*
- *Trypanosoma cruzi*
- *Leishmania chagasi*

Generally, the prognosis for bacterial or protozoal splenitis is better than that for fungal splenitis, although it depends on the etiological agent and specific variables (Ferri, et al., 2017).

The following are the other major splenic inflammatory conditions:

- **Hyperemic-hemorrhagic splenitis**. It is a tumefaction which occurs in forms of different severity, with varying degrees of volume modification and alteration of the red pulp (Marcato, 2015);
- **Hyperplastic splenitis**. pronounced splenomegaly due to excessive stimulation of the immunological functions of the lymphoid tissue and macrophage functions of splenic cords. It is a detectable finding in chronic infectious diseases and in chronic parasitosis, reaching its maximum development in protozoary blood diseases (Marcato, 2015), including Babesia spp., Ehrlichia canis and Anaplasma platys (Henning, et al., 2020);
- **Purulent and purulent-gangrenous splenitis**. Traumatic, hematogenous, or local extension forms of purulent inflammation can occur at the splenic level;

- **Necrotizing splenitis**;
- **Granulomatous splenitis**;
- **Diffuse chronic splenitis**. connected to the fibrous involution of the red pulp and follicles.

2.4.6 OTHER NON-NEOPLASTIC CONDITIONS

Splenic nodular hyperplasia. This disease is commonly seen in older dogs as an incidental finding. These nodules of increased consistency, which can be single or multiples and coalescing, tend to protrude from the surface of the spleen. Histologically, the nodules are composed by hyperplastic lymphoid cells or accumulations of erythroid, megakaryocytic, or myeloid cell (**Figure 2**). Unless they result in a hematoma, with the potential to break and generate a hemoperitoneum, they have no pathological effects. Differential diagnosis includes hematoma, hemangioma, hemangiosarcoma, primary or metastatic neoplasm (McGavin & Zachary, 2010).

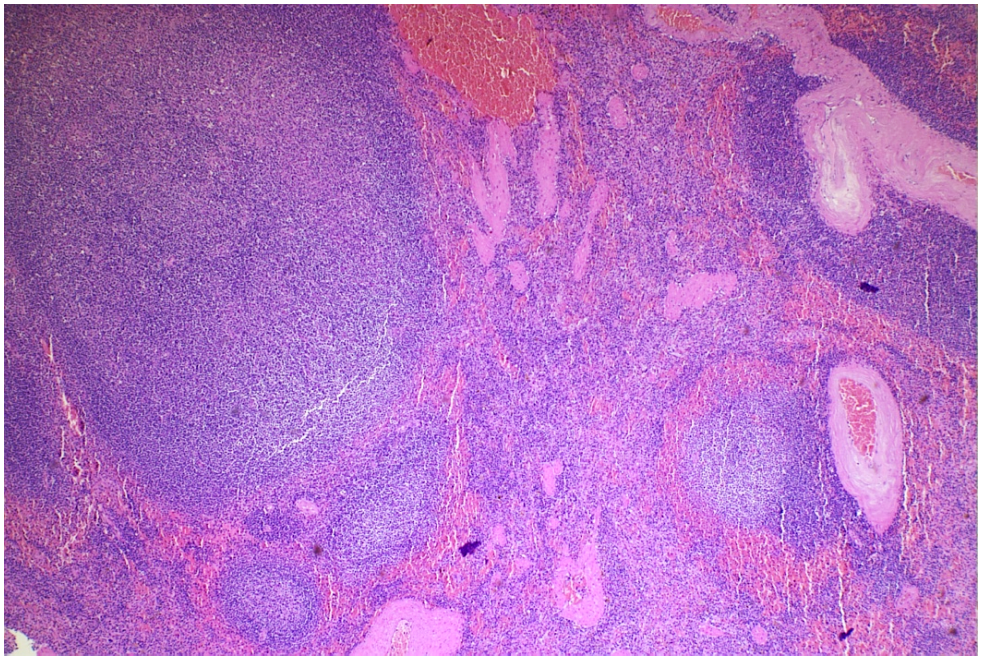

Figure 2 – *Splenic nodular hyperplasia (Hematoxylin-Eosin, 4x).*

Abscess. These are rare accidental findings, resulted from septicemia and/or bacteremia. Different sized abscesses can emerge from the splenic capsule. The exudate is often yellowish-white, though its composition and color might change depending on the cause (McGavin & Zachary, 2010), (**Figure 3**).

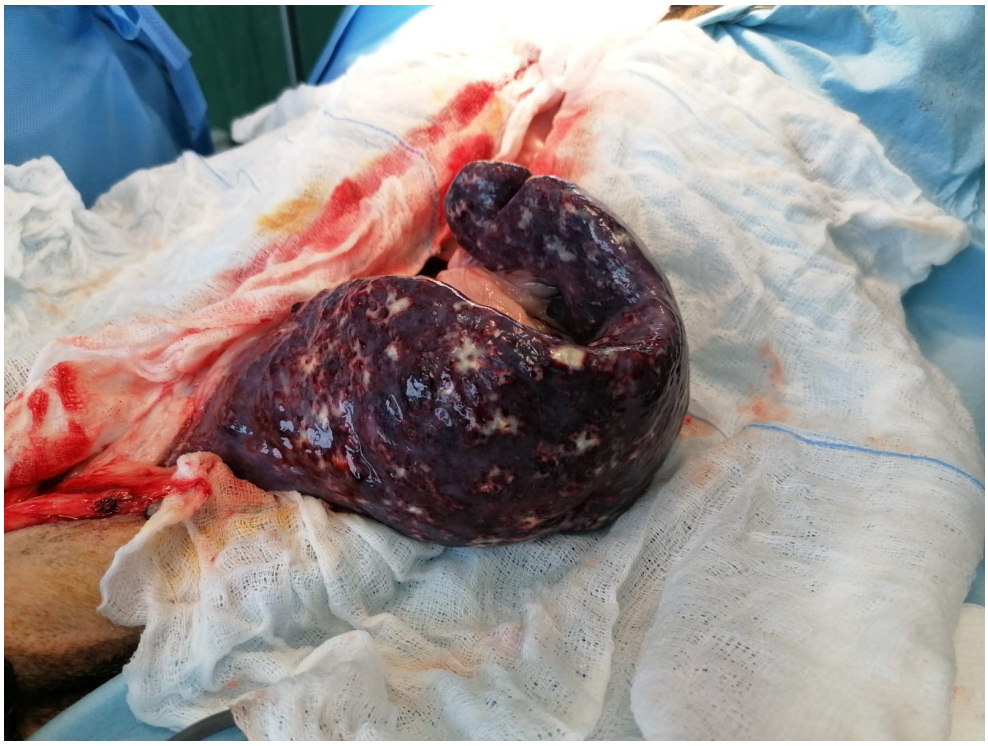

Figure 3 – *Multiple splenic abscesses. The surgery was performed at the Veterinary Teaching Hospital of the University of Parma.*

Extramedullary Hematopoiesis (*EMH*). Phenomenon where blood cells originate and grow outside of the bone marrow (Cordella, et al., 2020) to satisfy the demand for systemic circulation. EMH might be modest, frequently undetectable even under the microscope or it can be evidenced on the course of chronic anemia, splenic nodular hyperplasia, chronic respiratory failure or chronic cardiovascular disease. It is regarded as an accidental finding with no consequences for dogs because of the lack of clinics symptoms (Johns & Christopher, 2012). Myeloid, erythroid, and megakaryocytic lineages are typically involved, but usually only one type predominates (Marcato, 2015).

2.4.7 BENIGN NEOPLASIA

Fibrohistiocytic nodules. They are commonly identified, especially in dogs (**Figure 4**). They represent a heterogeneous group of diseases, both malignant and benign, which are included in different categories, including reactive histiocytosis (cutaneous and systemic) and histiocytic sarcoma (see chapter *Primary malignant tumors*), (Marcato, 2015). Dogs affected by this condition exhibit nonspecific symptoms such as anemia, neutrophilia and increased serum alkaline phosphatase (Moore, et al., 2012).

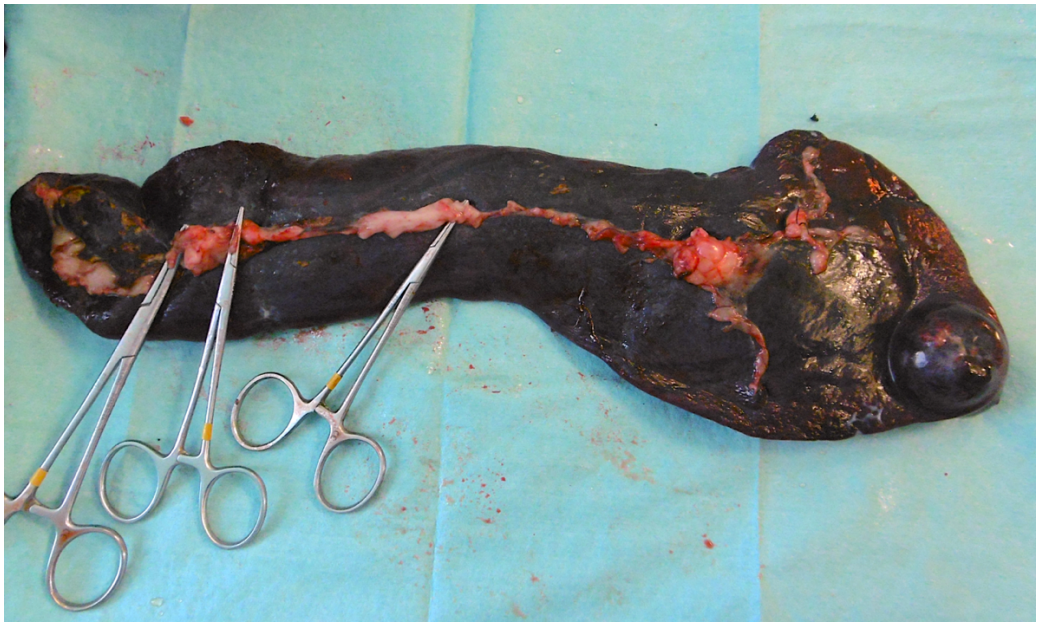

Figure 4 – *Fibrohistiocytic nodules*.

Myelolipoma. Benign neoplasm that appears as soft splenic nodules as a result of the accumulation of hematopoietic cells and adipose tissue (**Figure 5**). The spleen, liver and adrenal glands can all be impacted. Few cases of myelolipoma have been described in dogs, usually at splenic level; often, it is an incidental finding (Al-Rukibat & Bani Ismail, 2006). Furthermore, it tends to present as a single lesion, while multicentric myelolipomas are rare (Kamiie, et al., 2009). Contrary to other splenic lesions, ultrasonographically they appear as hyperechoic nodules with homogeneous echo structure; when fat is the primary component, they exhibit ultrasound patterns difficult to distinguish from lipoma (Carrillo, et al., 2012).

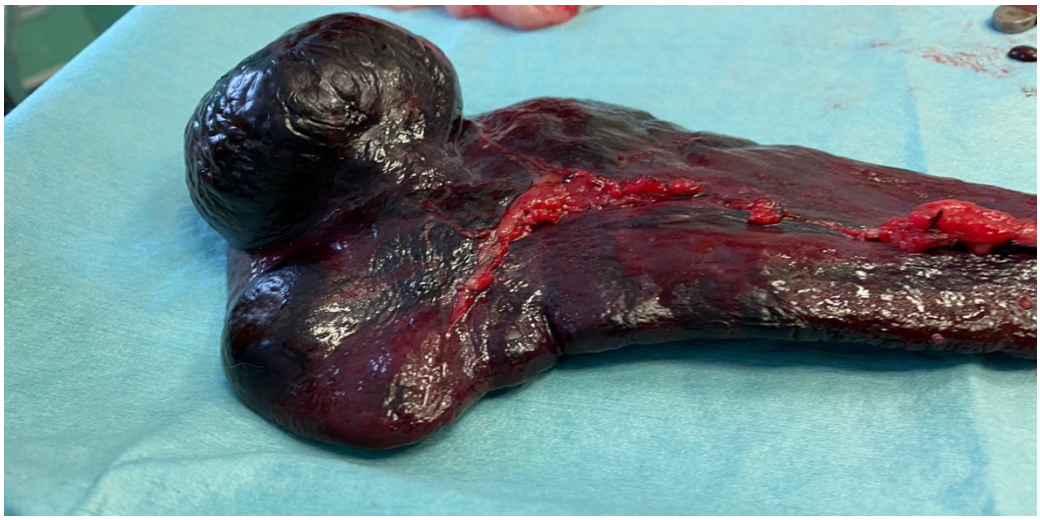

Figure 5 – *Myelolipoma and splenic hyperplasia nodules. The surgery was performed at the Veterinary Teaching Hospital of the University of Parma*.

Hemangioma. Blood artery endothelial cells are the origin of this sporadic benign neoplasm. The most common sites, in addition to the spleen, are the skin, tongue, synovium, conjunctiva, liver, kidney and spine.

It can be classified histologically as (Schöniger, et al., 2008):

- capillary
- cavernous
- arteriovenous
- lobular
- spindle cells
- epitheliod

The histological examination provides the definitive diagnosis, since the fine needle aspirate is typically too bloody, with some platelets and endothelial cells. Even though recurrence is quite common, surgery remains the preferred treatment (Marconato, et al., 2012).

Hematoma. It is generally associated with hyperplastic lymphoid nodules and is localized in the splenic parenchyma. The genesis is linked to the type of vascularization present in the marginal area of the white pulp, as the blood circulation is open. The mass can vary in size, and if the capsule bursts, haemoperitoneum may occur, with possible hypovolemic shock and circulatory failure (McGavin & Zachary, 2010), (**Figure 6**). The differential diagnosis with a hemangioma-hemangiosarcoma is difficult to reach macroscopically; therefore, a histological examination or the immunohistochemical demonstration of the antigen connected with factor VIII (a marker of

endothelial cells, identifiable in 90% of hemangiomas-hemangiosarcomas) are needed to obtain a diagnosis (Marconato, et al., 2012).

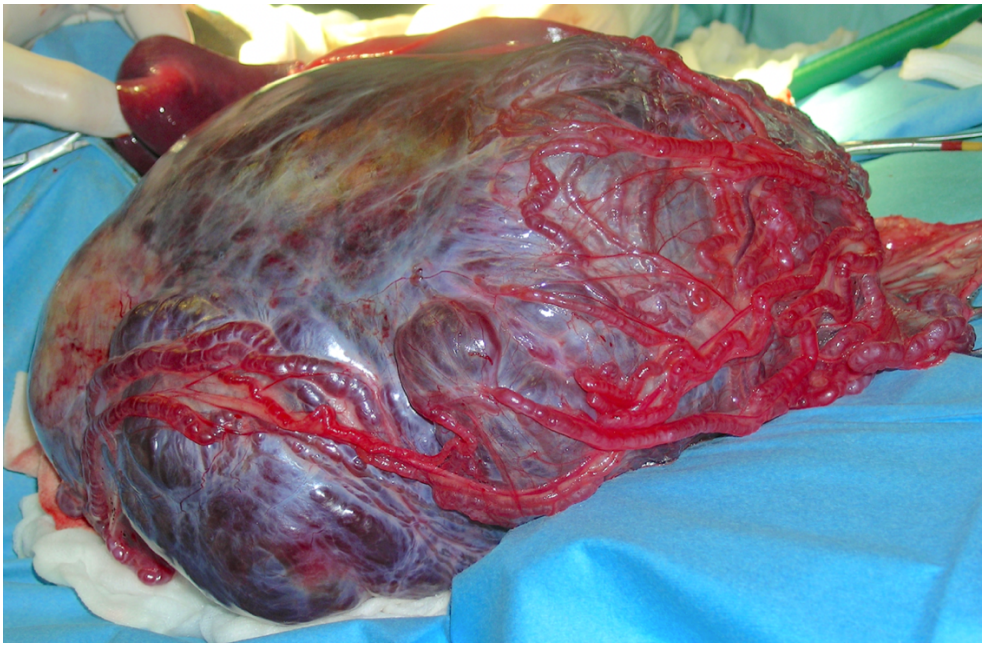

Figure 6 – *Splenic hematoma in extramedullary hematopoiesis*.

2.4.8 MALIGNANT PRIMARY TUMORS

Leiomyosarcoma. Leiomyosarcomas are mesenchymal-derived tumors that belong to the broad category of soft tissue sarcomas (*STS*), and are typically found in the abdominal cavity, retroperitoneally, or even at the uterine level (Aguilar, et al., 2013), (**Figure 7**). According to an article written by Linden et al. (2019) on abdominal STS, abdominal leiomyosarcomas had a prevalence of 38%.

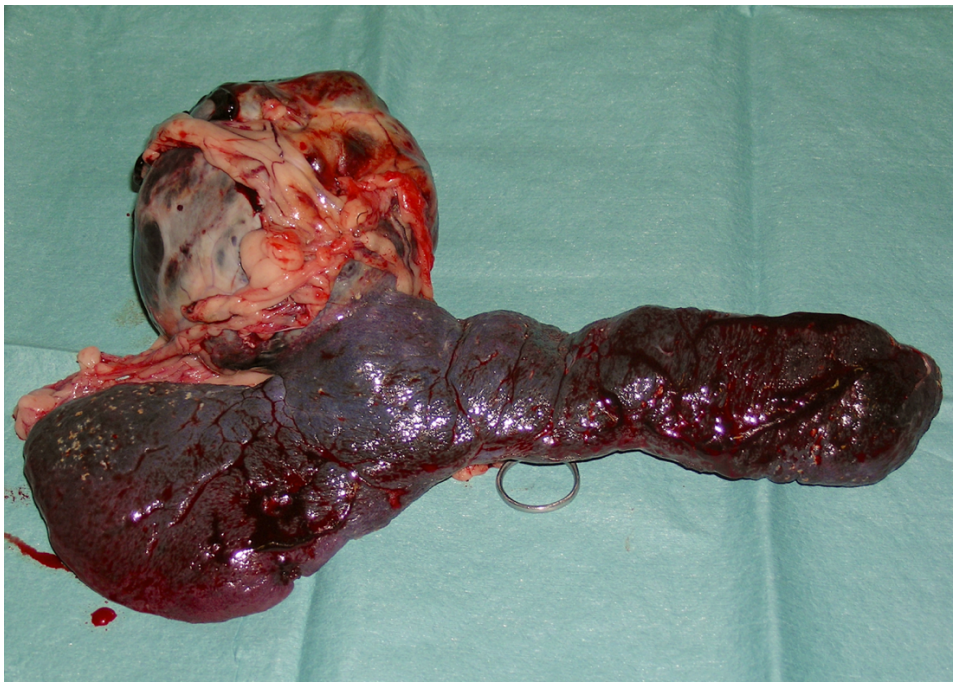

Figure 7 – *Splenic leiomyosarcoma.*

Liposarcoma. Occasionally discovered in visceral organs, liposarcoma is a mesenchymal tumor of the skin and subcutis that belongs to the macrocategory of STS (Withrow, et al., 2013). Biological behavior is locally invasive, infiltrating surrounding tissues (Baez, et al., 2004). When metastases do occur, they are typically discovered in the lung, liver, spleen, and bone.

Osteosarcoma. Extraskeletal osteosarcoma is a relatively uncommon malignant mesenchymal tumor that arises from viscera or soft tissues, characterized by the production of osteoid without involvement of the bone. It seems to occur in areas where there has been chronic damage or inflammation (Marconato, et al., 2012).

Malignant fibrous histiocytoma (*MFH*). It is an STS characterized by the proliferation of histiocytic and fibrous cells within the splenic red pulp (**Figure 8**). It mainly affects organs such as spleen, liver, lungs, hilar lymph nodes and mesenteric lymph nodes (Schmidt, et al., 1993), mainly in older dogs (Kerlin & Hendrick, 1996). In a study conducted by Waters et al. (1994) on different canine tumors, malignant fibrous histiocytoma accounted for 0.34% of all reported tumors (Kim, et al., 2018).

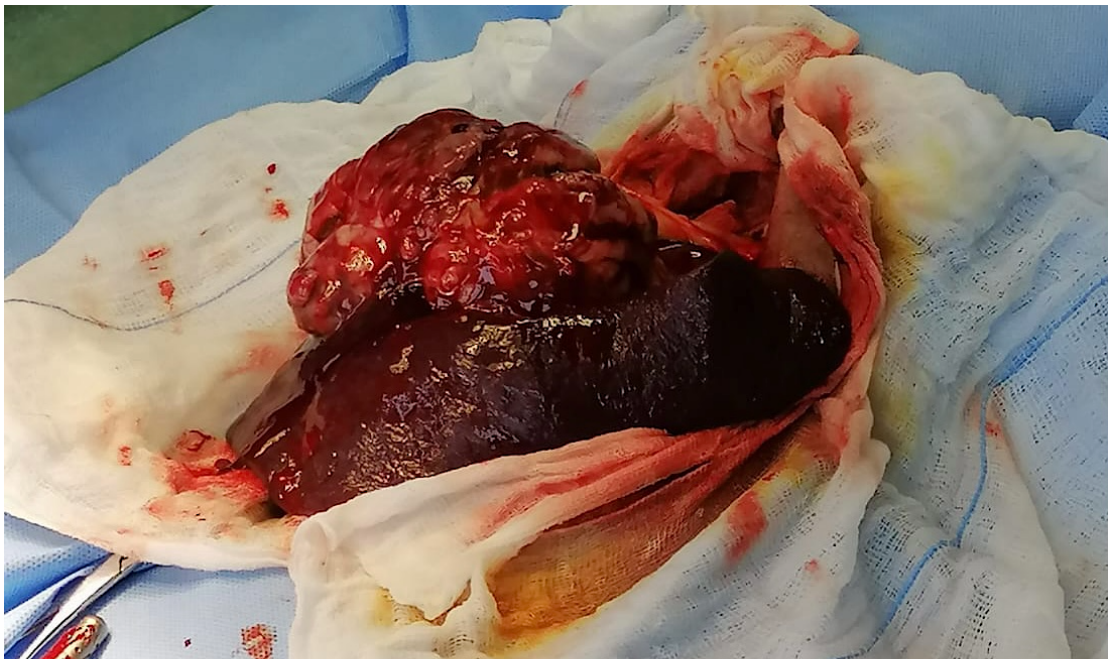

Figure 8 - *Malignant fibrous histiocytoma. The surgery was performed at the Veterinary Teaching Hospital of the University of Parma*.

Histiocytic sarcoma. It is an uncommon neoplasm, which originates from dendritic cells and accounts for less than 1% of malignant tumors of the lympho-reticular system (Dervisis et al., 2017), (**Figure 9**). This tumor occurs in a localized, disseminated or hemophagocytic form (Murray, et al., 2022). It occurs most frequently in lung, lymph nodes, liver, spleen, stomach, intestine, pancreas, mediastinum, skin and subcutaneous tissue, skeletal muscle, bone, joints, nasal cavities, and eye (Takahashi, et al., 2014). A particularly aggressive form, with a dismally poor prognosis, is known as *hemophagocytic histiocytic sarcoma* (Marconato, et al., 2012). This form also affects the liver, bone marrow, lung, and lymph nodes in addition to the spleen (Withrow, et al., 2013). Currently, no effective treatments have been described for hemophagocytic histiocytic sarcoma: in case of splenic localization, therapy involves splenectomy and adjuvant chemotherapy with lomustine, even if survival times are extremely short (Moore, et al., 2006).

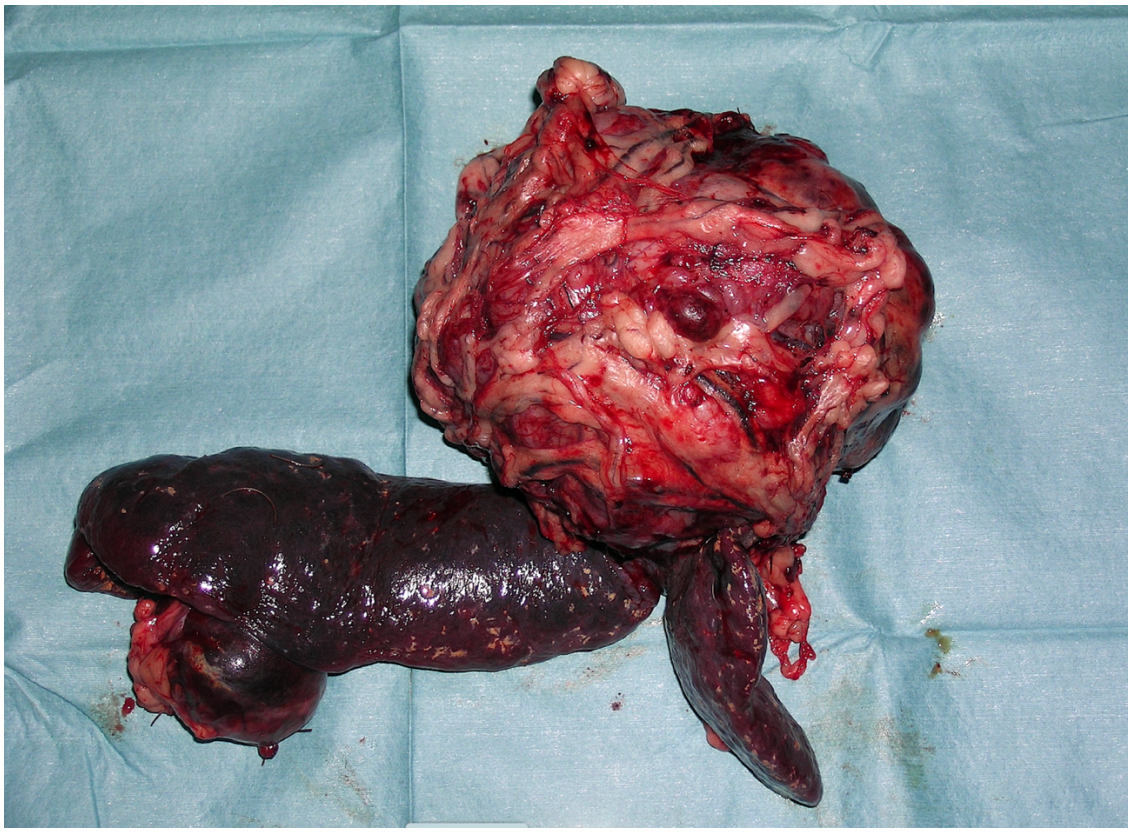

Figure 9 – *Canine histiocytic sarcoma*.

Fibrosarcoma. It is a mesenchymal-derived, locally invasive malignant tumor that belongs to the STS and is characterized by the presence of malignant fibroblasts. It can develop in any anatomical location; however, it is more common in skin, subcutis, and oral cavity (Withrow, et al., 2013); the spleen is less likely to be affected. According to statistics, it tends to affect older cats and dogs without sex or breed predilection; nevertheless, one study described that golden retrievers and Dobermann pinschers were more likely to develop fibrosarcoma than other types of soft tissue sarcoma, and another study reported that dogs with fibrosarcoma were significantly younger than dogs with other types of soft tissue sarcoma (Ettinger, et al., 2006).

Myxosarcoma. Metastasizing malignant tumor originating from primitive pleomorphic fibroblasts, with abundant myxoid matrix composed of mucopolysaccharides. According to Marconato et al. (2012), it is an uncommon tumor, mainly reported in adults or older dogs, it has infiltrative growth with indefinite boundaries (Goldschmidt & Hendrick, 2002). High cellularity and moderate nuclear pleomorphism are used as basis for differential diagnosis to myxoma (Marconato, et al., 2012).

Chondrosarcoma. It makes up around 10% of primary bone malignant lesions and it has a less aggressive behavior in extraskeletal location compared to extraskeletal osteosarcoma (Marconato, et al., 2012). Its etiology is unknown, although it appears to grow in dogs with pre-existing multiple cartilagineous exostoses (Doige, et al., 1978). It has an ordinarily low rate of metastasis; however, a more aggressive variant was described in 7 dogs, and its metastatic rate was 63% (Vinayak, et al., 2018).

Lymphoma. The most common round-cell tumor of the canine hematopoietic system (Marconato, et al., 2012), constituting 7 to 24% of all neoplasms in this species (Withrow, et al., 2013). It is characterized by an uncontrolled malignant lymphoid cell proliferation that primarily affects lymph nodes, but also the liver, the spleen, and other extralymphonodal sites, like the intestine, stomach, kidneys, or skin (**Figure 10**). It occurs mainly in adult and older dogs, although T-cell lymphoma tends to affect younger individuals (Ernst, et al., 2016).

According to the "*World Health Organization (WHO)*", there are 43 different varieties of B and T cell lymphomas; the marginal zone lymphoma, mantle cell lymphoma, B cell lymphoma, follicular lymphoma, peripheral T-cell lymphoma, and NK-cell lymphoma are the most important and frequently observed (Van Stee, et al., 2015). Marginal zone lymphoma is quite important at the splenic level.

The diagnosis and classification of splenic lymphoma are based on histopathological and immunohistochemical characteristics (Van Stee, et al., 2015).

On ultrasound, splenomegaly with irregular margins and hypoechoic, homogeneous or heterogeneous parenchyma can be observed. In many cases, it's possible to find small hypoechoic areas spread over the entire parenchyma, producing the characteristic "*honeycomb*" appearance (Marconato, et al., 2012).

Dogs having splenectomy can live for up to a year after surgery, and splenic B-cell lymphoma has a better prognosis in terms of treatment and prognosis (Van Stee, et al., 2015). Extranodal forms have a poorer prognosis than multi-center stage IV and V lymphomas (Zandvliet, 2016); these last, in fact, involve spleen and liver and have a prognosis of approximately 4-6 weeks without chemotherapy (MacEwen & Hurvitz, 1977), although there is significant variability.

After careful staging, it is generally recommended that, when the disease is shown to be confined in a single site, local therapies, such as surgery or radiotherapy, can be performed; whenever systemic progression is shown, the use of systemic therapies (e.g. chemotherapy) are required (Withrow, et al., 2013).

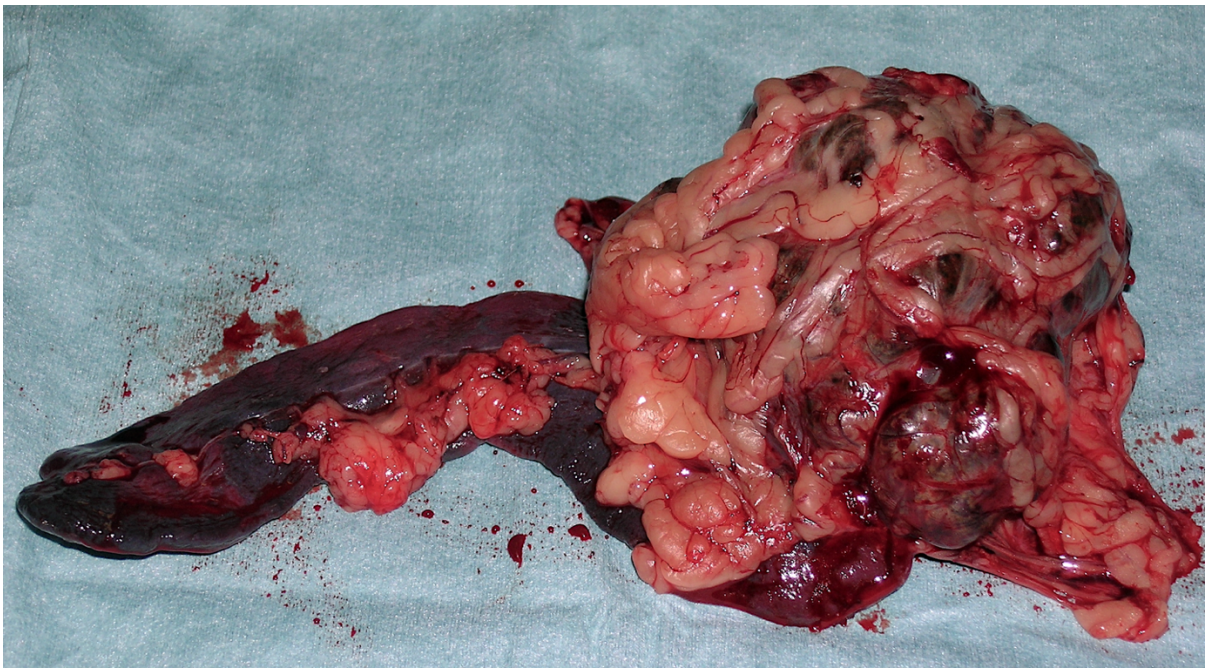

Figure 10 – *Splenic Lymphoma*.

Mastocytoma. Tumor originating from mast cells that can develop at the splenic level as a primary or metastatic tumor from skin or subcutaneous tissue. The biological behavior is often unpredictable, even if it seems to depend considerably on the site of onset: scrotal, perineal, preputial, auricular, digital, muzzle and muscle-cutaneous localizations are associated with a more aggressive biological behavior, with tendency to metastasize to other sites, such as skin, regional lymph nodes, spleen, liver, mesenteric lymph nodes and bone marrow. The undifferentiated cutaneous mast cell tumor usually precedes the visceral form; this last is more aggressive and usually represent a metastatic site (Marconato, et al., 2012).

Hemangiosarcoma (*HSA*). It is a common malignant tumor that develops from endothelial cells in dogs, where it accounts for 20% of mesenchymal tumors and 5-7% of all non-cutaneous neoplasms (Schultheiss, 2004).

Different studies confirmed the hypothesis of the so-called "*two-thirds rule*": approximately, 2/3 of the splenic masses are malignant and 2/3 of the latter are hemangiosarcoma; the remaining third of the splenic masses presumably are benign (Steve, et al., 2016; Davies & Taylor, 2020).

Canine hemangiosarcoma can affect any tissue in the body, although between 50 and 65% of all cases are found in the spleen (**Figures 11** and **12**). Hemangiosarcoma can also appear in the liver (9%), right atrium of the heart (from 3% to 25%), and, rarely, bones (particularly the proximal humerus, femur, ribs, and vertebrae) (Alexander, et al., 2019).

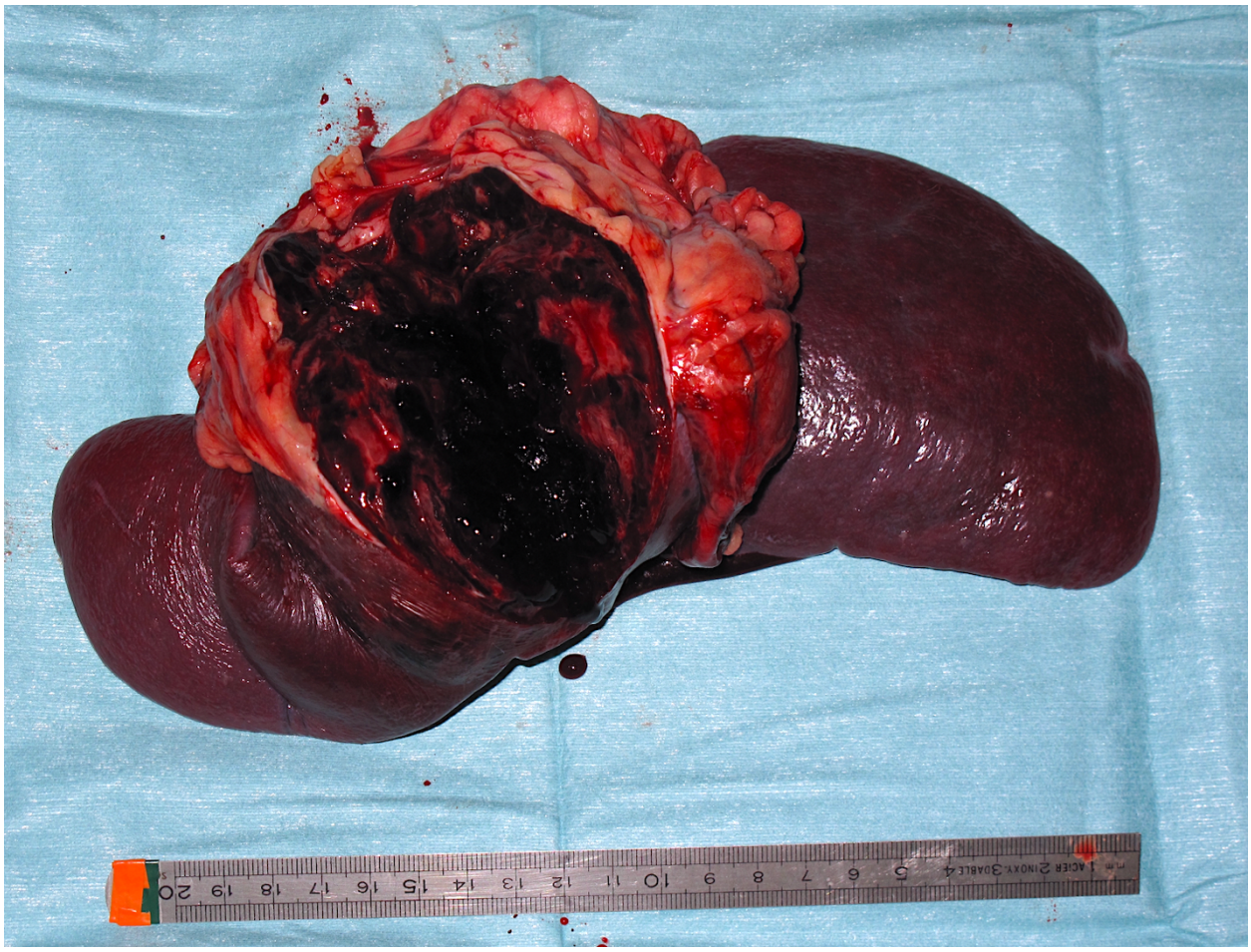

Figure 11 – *Ruptured splenic hemangiosarcoma.*

Figure 12 - *Spleen with splenic hemangiosarcoma (necropsy performed at the Department of Veterinary Sciences of the University of Parma).*

It is a highly metastatic tumor that grows quickly, particularly in the liver, lung, peritoneum, and central nervous system (Marconato, et al., 2019). Over 80% of dogs with clinical symptoms have distant metastases, and 25% of individuals with HSA had both splenic and atrial neoformations (Smith, 2003). It is typically associated with large-giant breed and older dogs, although sporadic cases in young dogs have been reported.

In dogs, the typical onset age ranges from 8 to 13 years.

Dog breeds that are considered predisposed are (Marconato, et al., 2012):

- German shepherd;
- golden retriever;
- pointer;
- boxer;
- Labrador retriever;
- English setter;
- great Dane;
- schnauzer;
- poodle;
- Siberian husky.

The precise histogenetic origin of HSA is still debated, and two hypotheses have been formulated: the first states that HSA originates from differentiated endothelial cells which, following several mutations, acquire neoplastic potential; the second hypothesis affirms that HSA originates from multipotent medullary cells (cancer stem cells), incompletely differentiated, which are close to the hemangioblast stage. From a molecular point of view, hemangioblasts express CD34, KIT (CD117) and VEGFR-2, while endothelial precursors express CD31 and factor VIII; thanks to these molecular studies, it is thought that HSA originates from bone marrow precursors stopped at the hemangioblast stage and, for this reason, they are classified among tumors with bone marrow origin (Marconato, et al., 2012).

An increased level of VEGF has been documented in the plasma of dogs with splenic HSA when compared with healthy dogs; in addition, serum endothelin-1 (a vasoactive pro-angiogenic peptide) was found to be higher in dogs with splenic HSA than in dogs with other splenic pathologies (Fukumoto, et al., 2015).

Hemangiosarcoma-related symptoms include signs of malaise and unexpected weakness. They are generally vague and frequently undiagnosed. However, acute collapse can happen after the original mass ruptures, leading to hemoperitoneum and hypovolemic shock (Wood, et al., 1998).

One of the most frequent complications of HSA is *disseminated intravascular coagulation* (*DIC*), characterized by widespread activation of coagulation, with intravascular deposition of fibrin and thrombotic occlusion of small and medium-sized vessels. This leads to a serious impairment of blood supply to various perfused organs and multi-organ failure (Levi & Cate, 1999).
The diagnostic process for a suspected HSA includes blood count, biochemical profile, coagulation profile, chest radiographs in 3 views, abdominal ultrasound and, in some cases, echocardiography (Vail, et al., 2020).
In the laboratory results, it is possible to observe a normocytic, normochromic, and regenerative anemia, along with thrombocytopenia and neutrophilic leukocytosis (which are secondary to a paraneoplastic syndrome or tumor necrosis, both of which are characterized by a shift to the left of the Arneth scheme). The latter is common in canines, particularly in 30-60% of dogs with splenic HSA (Grindem, et al., 1994). The blood smear reveals nucleated and polychromatophilic red blood cells, as well as poikilocytosis, acanthocytosis, anisocytosis, and schistocytosis, which are all present in 50% of dogs with splenic hemangiosarcoma (Hirsch et al., 1981). A "*leuko-erythroblastic reaction*" is the term used for the presence of young and immature neutrophils in association with nucleated red blood cells, a condition typically seen in dogs with HSA.

According to Marconato et al. (2012), the diagnosis must satisfy at least 4 of the following 6 criteria:

- Thrombocytopenia
- An increase by at least 25% in coagulation times (TP, APTT, ACT)
- Increased products derived from fibrinogen degradation
- Fragmentation of red blood cells
- Decrease in fibrinogen (< 80 mg/dl)
- Decrease in antithrombin III

According to Wood et al. (1998) and Withrow et al. (2013), clinical staging is a significant predictor of prognosis:

1. STAGE 1: splenic hemangiosarcoma is confined to the spleen without evidence of metastasis
2. STAGE 2: the tumor may have ruptured or may involve the regional lymph node
3. STAGE 3: presence of metastases

Primary Tumor (T)

T0: No evidence of tumor

T1: Tumor less than 5 cm diameter and confined to primary tissues

T2: Tumor 5 cm or grater or ruptured, invading subcutaneous tissues

T3: Tumor invading adjacent structures, including muscles

Regional Lymph Nodes (N)

N0: No regional lymph node involvement

N1: Regional lymph node involvement

N2: Distant lymph node involvement

Distant Metastasis (M)

M0: No evidence of distant metastasis

M1: Distant metastasis

STAGES

I: T0 or T1, N0, M0

II: T1 or T2, N0 or N1, M0

III: T2 or T3, N0, N1 or N2, M1

Chest X-ray examination may detect any pericardial or pleural effusions as well as potential lung or heart metastases. The most evident pulmonary pattern is nodular, with poorly defined nodules; occasionally, a diffuse alveolar pattern is also observed, due to pulmonary hemorrhages (**Figure 13**). One study found that the 3-view chest X-ray has a 78% sensitivity in detecting the presence of lung metastases compared to studies using only one or two views (Holt, et al., 1992).

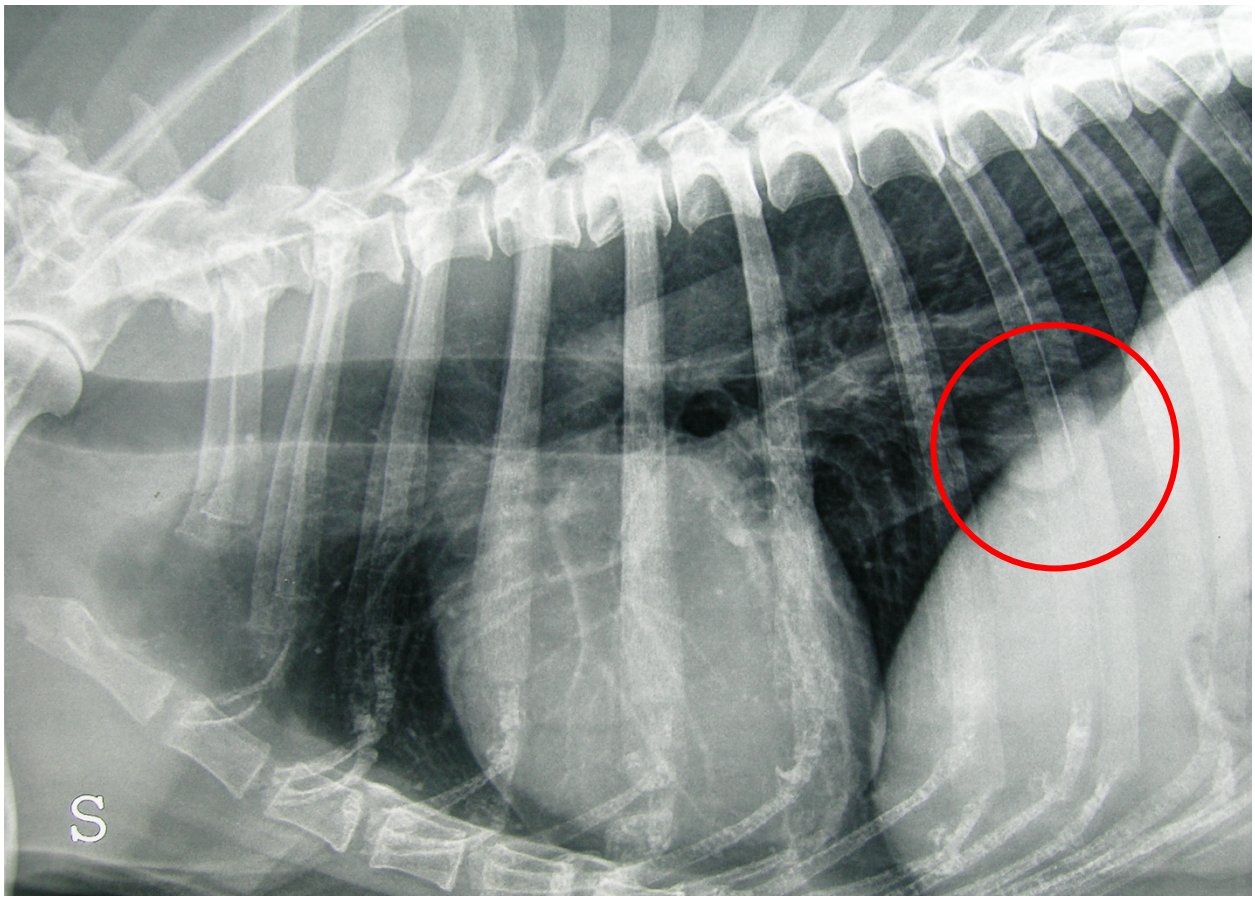

Figure 13 - *Chest radiograph of a patient with splenic hemangiosarcoma and pulmonary metastases*.

The *radiograph of the abdomen* allows to identify splenomegaly, splenic neoformations, and/or hemoperitoneum, which is defined as a pathological accumulation of blood or hemorrhagic effusion inside the peritoneal cavity (Lux, et al., 2013). However, it does not allow to obtain more detailed information on the structure of the organ parenchyma.

In addition to highlighting potential lymphadenomegaly, *abdominal ultrasound* can be useful for determining size, location, and number of splenic lesions. Macroscopically, it is impossible to determine the malignancy of a lesion exclusively by ultrasound because even hematomas and hyperplastic nodules can produce the same results (**Figure 14**). Frequently, HSA contains necrotic and hemorrhagic areas that appear as anechoic or complex mass areas. Splenic metastases are often characterized by the existence of nodules with a target appearance, which have a hyperechoic center and a hypoechoic peripheral perimeter (Marconato, et al., 2012). Additionally, any distant nodules or metastases, such as those in the liver or peritoneum, might be identified by abdominal ultrasonography.

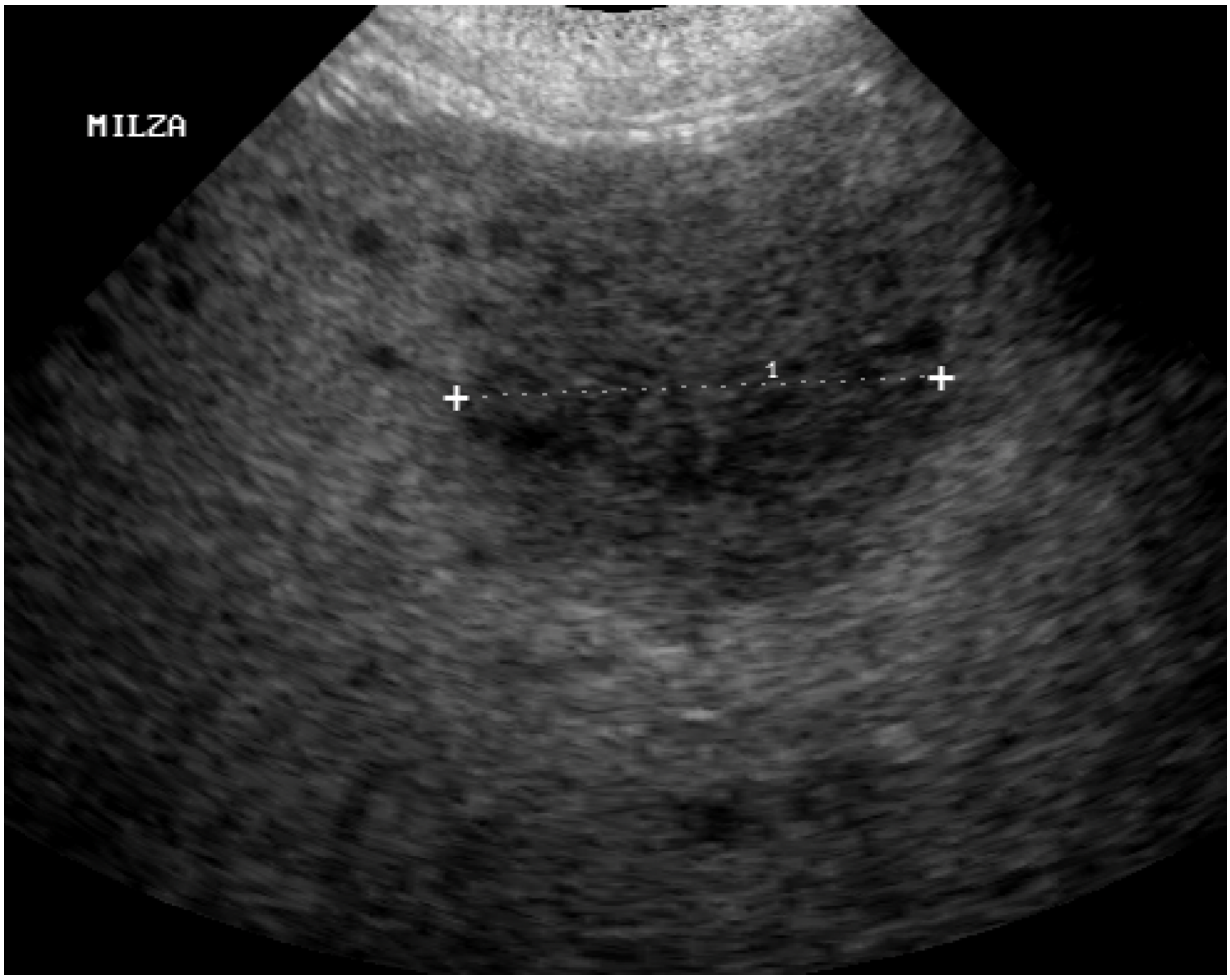

Figure 14 - *Abdominal ultrasound of a splenic hemangiosarcoma*.

Due to the organ fragility, *ultrasound-guided cytological examination* of the spleen can occasionally be challenging, particularly in cases of big nodules, the rupture of which could result in the spread of tumor cells along the needle path or in the abdomen.
Furthermore, HSA does not always exfoliate; therefore, the samples are often contaminated with abundant blood and lacking in diagnostic cells (Marconato, et al., 2012).

Complete staging is possible with *magnetic resonance imaging* (*MRI*) or *computed tomography* (*CT*), especially when looking for metastases (**Figure 15**). Their integration into the routine for surgical planning, metastasis research or staging improves the accuracy of prognosis: in particular, they aid in better defining the anatomical origin, the extent of the lesions and the early identification of lung metastases (Withrow, et al., 2013).

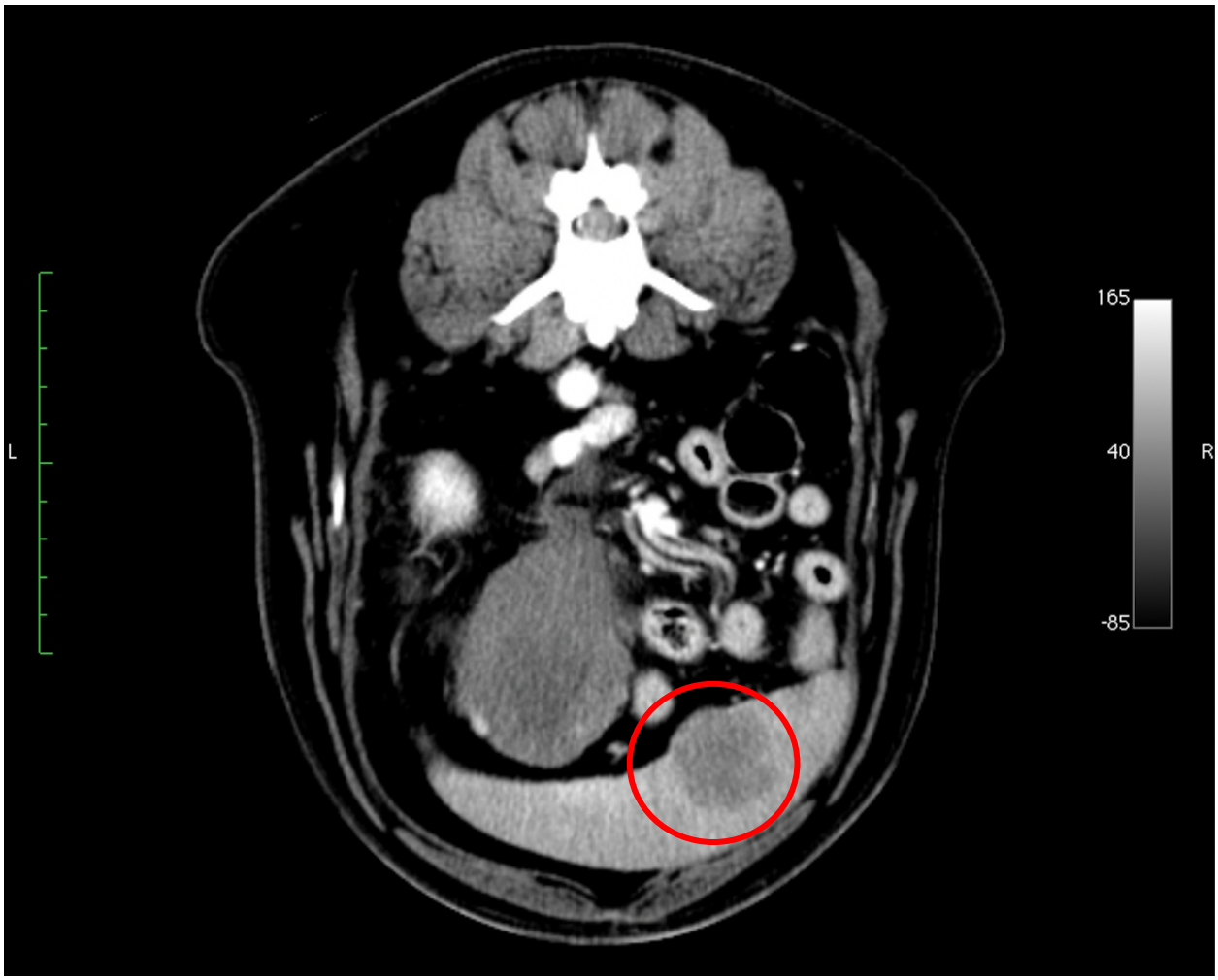

Figure 15 - *Total body CT scan of a patient with splenic hemangiosarcoma.*

To reach the final diagnosis, histological examination is the gold standard, provided that the examined sample is suitable and reliable; ideally, the entire organ should be sent to the laboratory, so that pathologists can gather more samples and have a greater chance of diagnosis. If this is not possible, it is advisable to send more portions of the spleen, avoiding particularly necrotic and hemorrhagic areas (**Figures 16** and **17**). Histologically, it is possible to observe atypical oval and/or spindle cell elements, with anisocytosis and anisocariosis aspects with a high mitotic index. The neoplastic endothelial cells organize themselves to imperfectly delimit vascular channels as they lay on a thin layer of collagen fibers (Marconato, et al., 2012).

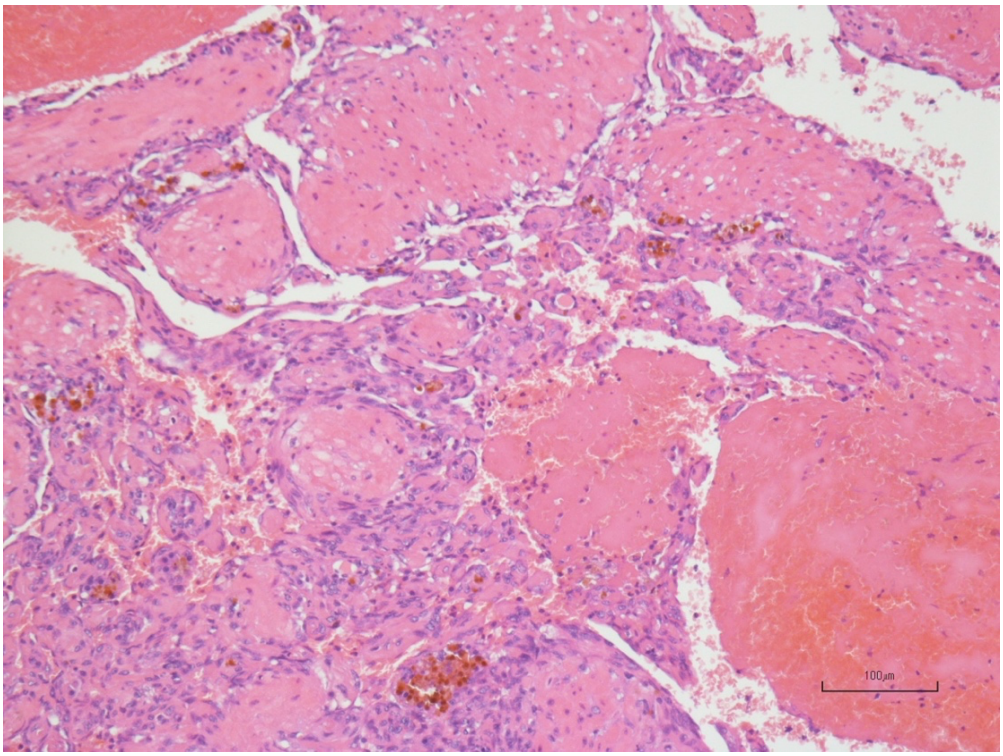

Figure 16 - *Histological image of splenic tissue with final diagnosis of splenic hemangiosarcoma (hematoxylin-eosin staining - 4x).*

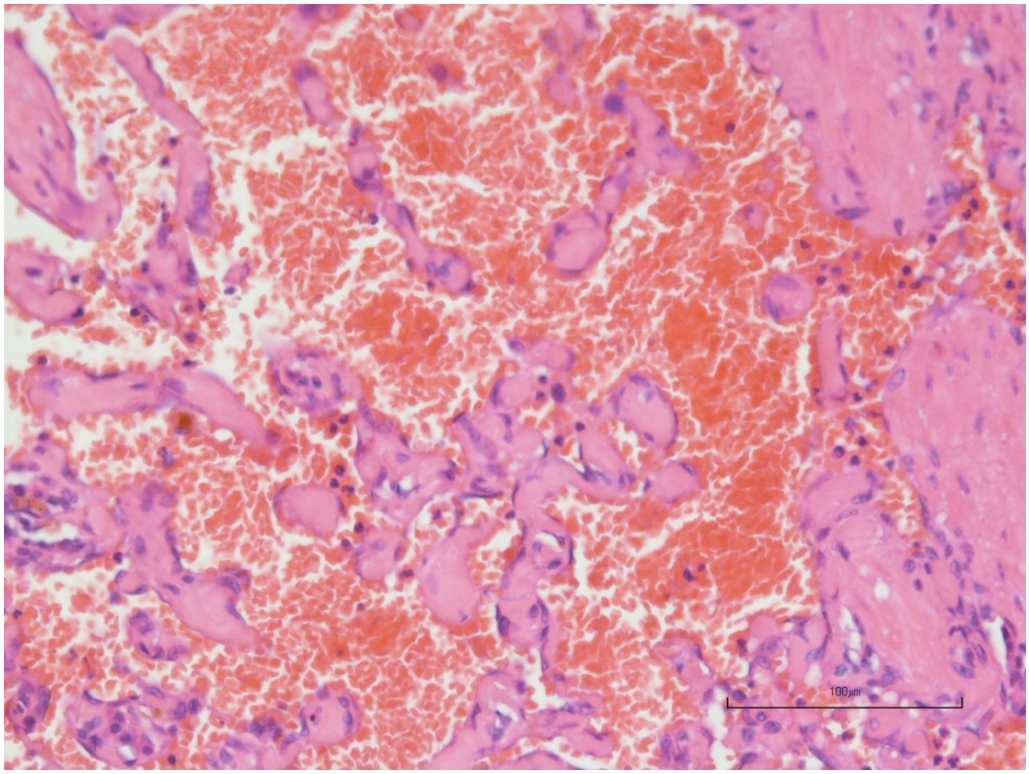

Figure 17 – *Histological image of splenic tissue with final diagnosis of splenic hemangiosarcoma (hematoxylin-eosin staining – 10x).*

The *therapy* consists of:

- splenectomy (in absence of diffuse lung metastases);
- chemotherapy with doxorubicin, used as a single agent or in combination with ifosfamide, vincristine or cyclophosphamide (Finotello, et al., 2017); as an alternative, metronomic chemotherapy can be used;
- immunotherapy.

The literature suggests that doxorubicin, both as an adjuvant and as a neoadjuvant, is the most active chemotherapeutical drug against HSA. Besides that, there are protocols that combine doxorubicin with additional chemotherapeutical drugs, such as cyclophosphamide, minocycline, vincristine, methotrexate, dacarbazine, ifosfamide, and deracoxib (Withrow, et al., 2013); however, the mentioned above survival time does not significantly change in these protocols.

Due to the vascular nature of the tumor, *metronomic chemotherapy* can be an alternative strategy. One study reported similar results for patients with stage II splenic HSA treated with splenectomy, piroxicam and metronomic chemotherapy (with alternating cycles of cyclophosphamide and etoposide) compared to dogs who have only undergone cycles of doxorubicin (Lana, et al., 2007), although other studies have not found the efficacy of this treatment (Withrow, et al., 2013).
Although *immunotherapy* is not frequently used to treat HSA in the clinical setting, studies have shown that it may be effective and offer a new approach to cancer treatment. In fact, one study demonstrated that a vaccine was effective in eliciting a humoral immune response in dogs receiving concurrent doxorubicin (U'Ren, et al., 2007); another study evaluated the adjuvant use of *muramyl tripeptide phosphatidylethanolamine* encapsulated in liposomes (*L-MTP-PE*). In particular, dogs receiving splenectomy, systemic chemotherapy, and L-MTP-PE immunotherapy had a median survival time of 273 days (Vail, et al., 1995). Although it has been approved in the European Union for the treatment of human osteosarcomas, L-MTP-PE is not yet commercially available, so there are few studies on it (Withrow, et al., 2013).

Due to tumor location into visceral organs and its high metastatic potential, *radiotherapy* is not frequently employed to treat splenic hemangiosarcomas (Withrow, et al., 2013).

The prognosis of canine hemangiosarcoma depends on tumor location, clinical stage, and treatment option. Ventricular arrhythmias, splenic rupture, and hemoperitoneum may be poor prognostic indicators.

Unfortunately, visceral hemangiosarcoma has a silent evolution even for long periods, accompanied by non-specific symptoms; the detection of this tumor occurs late, or even when the neoplasm is already metastatic, so the treatment is often only palliative (Thamm, 2013).
The *median survival time* (*MST*) is low for the majority of primary splenic hemangiosarcoma cases, despite surgery, chemotherapy, or radiotherapy; one-year survival is less than 10%. (Withrow, et al., 2013).

Due to the significant metastatic rate of this tumor, the prognosis for dogs with splenic HSA receiving only surgical treatment is quite negative, with a median survival time ranging between 19 and 86 days (Spangler & Kass, 1997). A median survival time of 5-7 months is possible with the inclusion of adjuvant chemotherapy (Kahn, et al., 2013).

Tumor staging plays a fundamental role, since a stage 1 splenic hemangiosarcoma has a better prognosis (*MST* of 239-355 days) than a stage 2 hemangiosarcoma (*MST* of 120-148 days) (Vail, et al., 1995) or a stage 3, in which median survival times range from 23 to 40 days (Lucroy, et al., 2020).

3. AIM OF THE STUDY

Splenic diseases have different manifestations, and they can have neoplastic or non-neoplastic characteristics. The spleen can be affected by both primary and secondary pathologies and, regardless of the etiology, these may be very difficult to distinguish grossly (Bichard & Sherding, 2009) although the prognosis may vary considerably.
Canine splenic hemangiosarcoma is an extremely aggressive neoplasm with a poor prognosis due to its high metastatic rate. The aggressiveness and heterogeneity that characterize this tumor represent a diagnostic and therapeutic challenge for both clinicians and pathologists.

The main objective of this experimental thesis was to review the literature about splenic diseases and to compare these data with those obtained at the Veterinary Teaching Hospital of the University of Parma from dogs undergone splenectomy from January 2014 to April 2022.
In particular, the survival time (*ST*) of each patient were recorded, to determine whether signaling, the existence of comorbidities or the occurrence of abdominal bleeding may have had an impact on the survival time. Furthermore, the impact of hemoperitoneum, regardless of the histological diagnosis, on the survival dogs was also evaluated.

4. MATERIALS AND METHODS

The retrospective clinical study was conducted on dogs led to the emergency service of the Veterinary Teaching Hospital of the Department of Medical-Veterinary Sciences of the University of Parma or undergone elective surgery for a splenic mass at the same Department.

The study included dogs that had surgery between January 2014 and April 2022, after a suspected or confirmed diagnosis of splenic neoformation with or without the presence of hemoabdomen.

Data collection was carried out using keywords related to the numerous instances during these 8 years, such as "*splenectomy*" or "*spleen*" in the database software used at the hospital, and from dogs directly seen by the author.

To be included in this study, dogs must satisfy the following requirements:

- diagnosis of a suspected or confirmed splenic mass;
- radiographic examination and abdominal focused assessment by sonography at the admission;
- urgent or planned splenectomy;
- presence of a report of the spleen's histological examination;
- absence of concomitant metastases;

Two dogs were excluded because of splenectomy after *GDV* and after splenic torsion not related to a concomitant splenic neoformation.

All patients not complying with the inclusion criteria were excluded from the study.

During the general physical examination, the following parameters were analyzed: weight, mucous membrane color, capillary filling time (*TRC*), sensory state, body temperature, heart rate and auscultation, respiratory rate, and abdomen palpation.
Collateral exams were then conducted, including radiographic examinations in two or three projections, abdominal ultrasound examination and, total body CT (*Computer Tomography*) for dogs enrolled in the last years. A fine-needle aspiration biopsy (*FNA*) of spleen and liver under ultrasound guidance was carried out on 5 individuals, in order to determine the possible origin of the neoformation and the existence or absence of liver metastases

4.1 STUDY METHODOLOGY

The following information was collected for each individual patient: age, date of birth, sex (Female - F/Male - M/Sterilized Female - SF/Castrated Male - CM), breed, weight, presence of concurrent pathologies, presence or absence of hemoabdomen; reports of abdominal ultrasound, chest X-ray, CT, or FNA of liver and spleen; date of splenectomy; presence or absence of intra-operative complications and the potential necessity of using intra- or post-surgery blood derivatives; histological diagnosis with reference to the laboratory that analyzed the samples and possible immunohistochemical diagnosis; follow-up including presence/absence of distant metastases; outcome of the dog with potential date of death, *Time to progression* (*TTP* - time, expressed in days, which goes from the date of surgery to the diagnosis of metastasis) and *Survival time* (time, expressed in days, which goes from the date of surgery to the day of death or to the last day of this study, indicated as June 30th 2022), (**Table 1**) .

All dog owners were contacted by telephone to collect the most recent history and survival status of each dog included.

PARAMETERS
REPORTING AND HISTORY
- ID
- Dog name
- Age (years)
- Sex (M/F/SF/CM)
- Breed
- Weight (Kg)
- Concomitant pathology (Yes/No)
- Concomitant pathology (Which)
- Hemoabdomen (Yes/No)
STAGING
- Abdominal ultrasound
- Chest X-Ray
- CT
- FNA
SURGERY
- Surgery date
- Gastropexy (Yes/No)
- Intra-operative complications (Yes/No)
- Intra-operative complications (Which)
- Blood derivatives (0 no, 1 concentrated red blood cells, 2 blood, 3 plasma, 4 autotransfusion)
HISTOLOGY
- Diagnosis
- Immunohistochemistry (Yes/No)
- Immunohistochemistry (What)

THERAPY
- Chemotherapy (Yes/No)
- Chemiotherapy protocol
FOLLOW-UP
- Distant metastasis (Yes/No)
- Distant metastasis (site)
- Distant metastasis (date)
OUTCOME
- Death (Yes/No)
- Disease-related death (Yes/No)
- Lost to follow up (Yes/No)
- Date of death or last medial visit (dd/mm/yy)
- Time to progression
- Survival time

Table 1 – *Data included in the Excel worksheet.*

4.2 STATISTICAL METHOD

Microsoft Excel and *R-software* were used for statistical analysis.

The median age and median weight of the subjects were calculated by *Microsoft Excel*, and the corresponding graphs were created.

Kaplan-Meier Survival curves were modeled based on the available data using two R software packages, named *Survival and Survminer* (R Development Core Team, 2011).

Based on the Log-Rank test, the presence of statistically significant differences in terms of survival probability between different groups were evaluated. In the *Log-Rank test*, the null hypothesis was that there was no difference in survival between the different groups. The Log-Rank test is a non-parametric test, which makes no assumptions about the survival distributions. Essentially, the Log-Rank test compares the observed number of events in each group to what would be expected if the null hypothesis were true (i.e., if the survival curves were identical).

The *Chi-square test* for goodness of fit was used to compare the observed distribution to the expected distribution based on literature (Spangler, et al., 1997).

Median Survival Time (*MST*) was defined as the number of days between splenectomy and death, or the conclusion of the clinical study, set on June 30th 2022. When the dog owner did not precisely remember the date of death, the latter was set for the 15th day of the month of death.
Time to Progression was defined as the time (expressed in days) between the day of surgery and the day of ascertained occurrence of metastases, also known as disease-free time.
A p.value < 0.05 was considered significant.

5. CASE STUDY

CASE N°	AGE (years)	SEX	WEIGHT (Kg)	DIAGNOSIS	STATUS	ST (days)
1	10	M	45	Hemangiosarcoma	D	27
2	5	F	34	Hemangioma	D*	1179
3	10	M	20	Fibrohistiocytic nodules	D	535
4	14	F	13	Extramedullary hematopoiesis	L	1148
5	9	M	42	Fibrohistiocytic nodules	D*	489
6	6	F	35	Pyogranulomatous splenitis	ND	-
7	9	F	12	Fibrosarcoma	D*	958
8	6	F	25	Nodular hyperplasia	L	2071
9	13	M	41	Nodular hyperplasia	D*	946
10	12	F	25	Extramedullary hematopoiesis	ND	-
11	7	M	9	Fibrohistiocytic nodules	A	2025
12	7	F	9	Lymphoid hyperplasia	A	1653
13	7	M	25	Fibrohistiocytic nodules	A	1653
14	15	F	5	Hemangiosarcoma	D	138
15	12	SF	23	Hematoma	D*	504
16	14	M	40	Hematoma	L	1543
17	7	F	17	Hemangiosarcoma	D	1023
18	8	F	37	Hemangiosarcoma	D	198
19	7	CM	43	Hemangiosarcoma	D	109
20	8	SF	27	Hemangiosarcoma	D*	599

CASE N°	AGE (years)	SEX	WEIGHT (Kg)	DIAGNOSIS	STATUS	ST (days)
21	12	SF	7	Hemangiosarcoma	D	161
22	10	F	8	Large cells lymphoma	L	1290
23	12	M	20	Fibrohistiocytic nodules	A	904
24	12	F	11	Hematoma	A	898
25	10	M	20	Fibrohistiocytic nodules	D*	635
26	10	M	40	Abscess	D*	563
27	1	F	18	Passive congestion	A	876
28	9	F	12	Fibrohistiocytic nodules	A	777
29	10	M	39	Mixosarcoma	D	67
30	14	F	25	Extramedullary myleopoiesis	D*	84
31	12	M	25	Splenic follicular lymphoma	D	57
32	11	F	22	Fibrohistiocytic nodules	D*	60
33	10	M	37	Passive congestion	ND	-
34	11	M	8	Myelolipoma	D*	2
35	6	M	10	Passive hyperemia	D*	501
36	7	M	40	Hemangiosarcoma	D	1
37	12	F	21	Myelolipoma	A	423
38	21	F	25	Hematoma	D	1
39	12	M	34	Hemangiosarcoma	D	1
40	12	F	44	Nodular hyperplasia	A	318
41	10	F	31	Hematoma	L	366

CASE N°	AGE (years)	SEX	WEIGHT (Kg)	DIAGNOSIS	STATUS	ST (days)
42	9	SF	9	Malignant fibrous histiocytoma	D	116
43	17	M	12	Perineural sarcoma	D	116
44	8	M	18	Hematoma	A	269
45	7	M	32	Nodular hyperplasia	A	262
46	14	F	36	Hematoma	A	207
47	9	SF	32	Myelolipoma	A	223
48	7	SF	17	Myelolipoma	A	161
49	11	SF	35	Hemangiosarcoma	D	111
50	9	M	39	Undifferentiated sarcoma	A	126
51	11	M	29	Hematoma	A	103
52	13	SF	14	Nodular hypeprlasia	A	108
53	10	F	35	Hematoma	A	99
54	12	M	11	Nodular hyperplasia	A	85

Table 2 - *List of the 54 cases included in the study. "**D**" stands for "Dead", "**D***". stands for "Dead by other causes", "**A**" stands for "Alive", "**L**" stands for "Lost to follow-up", "**ND**" stands for "Not-Defined" referred to the 3 dogs that didn't satisfy the aforementioned inclusion criteria.*

6. RESULTS

Results will be analyzed talking first about splenic neoformations in general, then going into the specific about splenic hemangiosarcoma.

A follow-up could be obtained for 46 of the 54 dogs in the study. The remaining 8 dogs were not included because 5 dogs lacked a follow-up, 1 had splenectomy after gastric torsion, 1 had after splenic torsion, and 1 had concurrent multicentric lymphoma that was already present at the time of the surgery.

Twenty-three dogs were males (42%), 22 dogs were females (40%), 8 female dogs were spayed (15%) and one male dog was neutered (2%) (**Figure 18**).

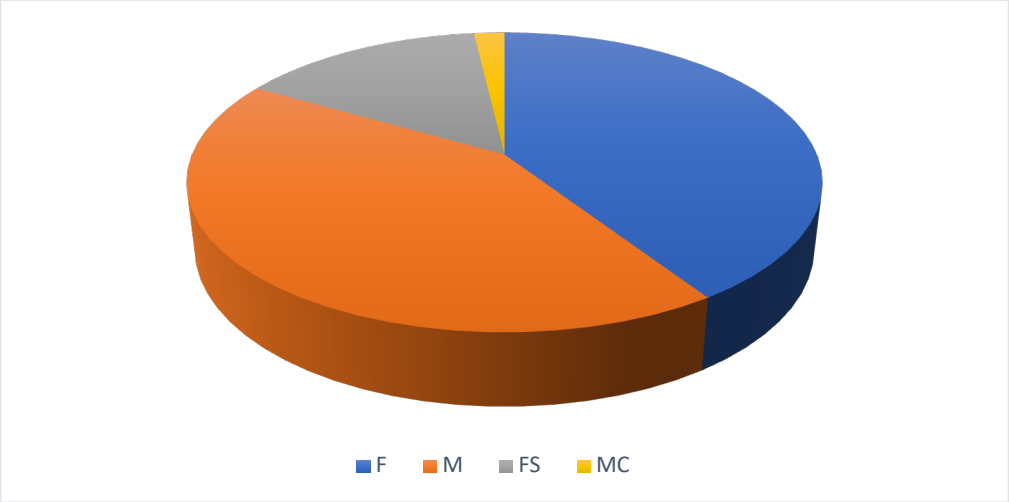

Figure 18 - *Sex distribution of patients undergoing splenectomy*.

When statistically comparing the effect of sex on survival, it was observed that 50% of males had a survival probability of 500 days following surgery, compared to 900 days reached by females (**Figure 19**). This difference was not statistically significant ($p = 0.19$) but showed a trend for a longer survival for females.

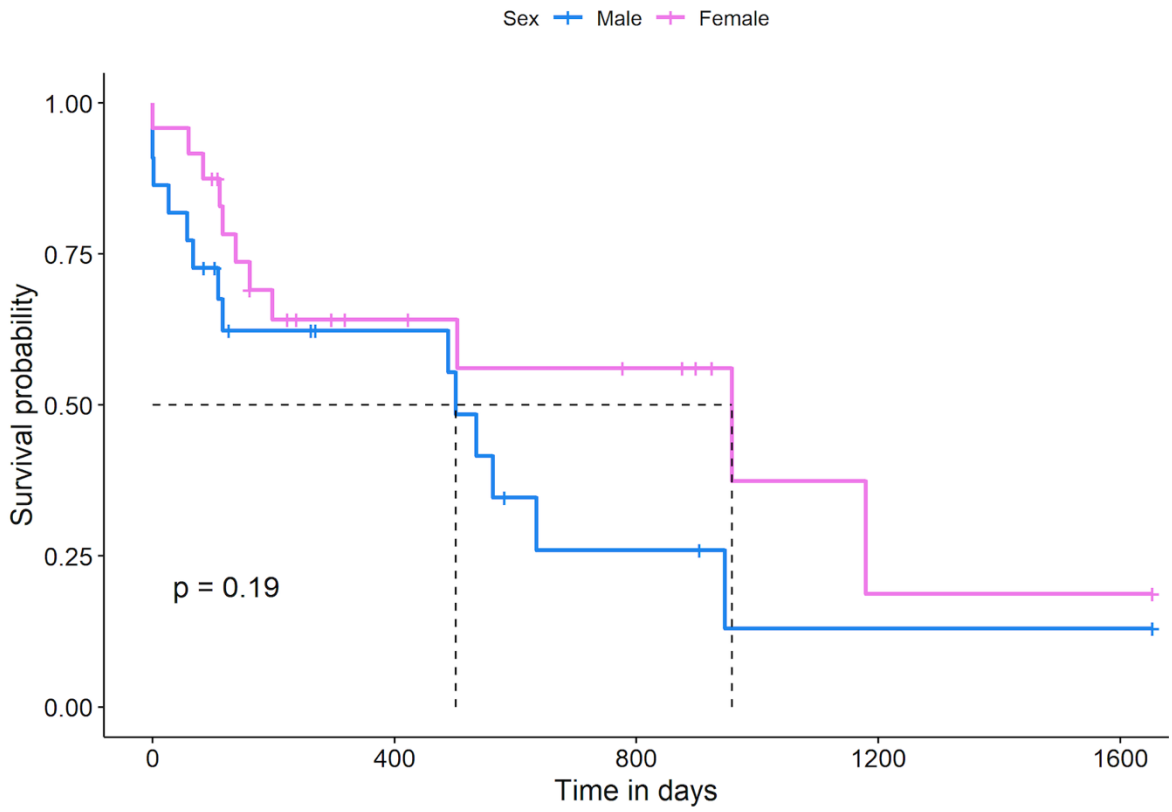

Figura 19 – *sex effect on Keplan-Meier curve*.

The median age was 10 years (range from 1 to 21 years). The oldest patient was 21 years old at the time of splenectomy and the final histological diagnosis was hematoma and extramedullary hematopoiesis; the younger patient was 1 year old and the histological diagnosis revealed the presence of passive congestion.

According to ENCI classification, dogs breed included were:

- Mixed breed < 20 kg (n = 11);
- Mixed breed > 20 kg (n = 6);
- Shepherd dog (n = 9);
- Molosser (n = 5);
- Brachycephalic (n = 6);
- Pointing dog (n = 2);
- Retrievers (n = 6);
- Terrier (n = 2);
- Pincher and Schnauzer (n = 2);
- Spitz-type dog (n = 1);
- Dachshund (n = 1).

Although some macro-differences can be graphically shown, the statistical analysis does not show any significant differences in survival according to breed (**Figure 20**).

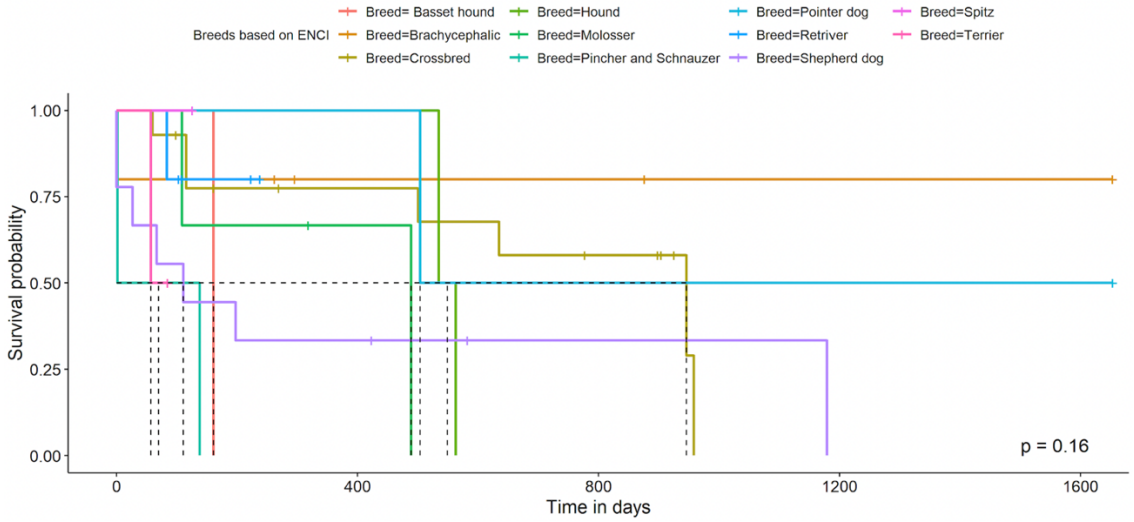

Figure 20 – *Breed effect on Kaplan-Meier curve.*

Twenty-six out of 54 dogs (53.5%) had concomitant diseases discovered during surgery and 5 dogs (9.2%) presented tumors in other locations diagnosed after splenectomy, in particular:

- plasmacytoma (n=1);
- uterine leiomyoma (n=1);
- solid apocrine gland anal sac carcinoma metastatic to the iliac lymph nodes (n=2);
- recurrence of adenocarcinoma of the hepatoid glands (n=1).

From the statistical analysis (**Figure 21**), comorbidities did not significantly influence survival (p = 0.25).

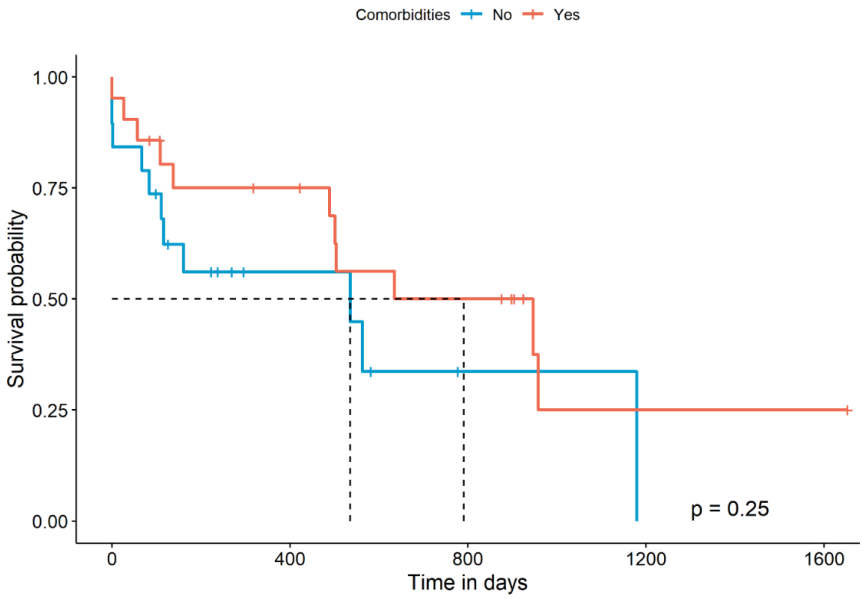

Figure 21 – *Kaplan-Meier curve based on presence/absence of comorbidities.*

Similarly, when focusing on the presence of neoplastic comorbidities (**Figure 22**), no significant differences in terms of survival probability were found (p = 0.36).

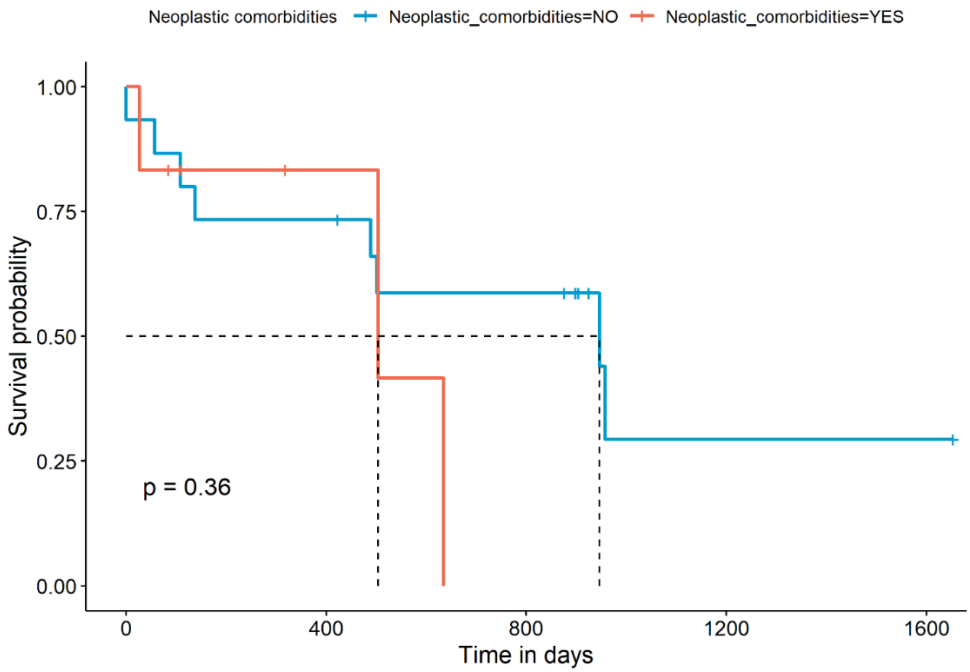

Figure 22 – *Kaplan-Meier curve based on presence/absence on neoplastic comorbidities*.

All dogs underwent abdominal ultrasonography and chest radiographs in three views (DV or VD, and LL); only 3 dogs (5.5%) underwent total body CT, and 5 dogs underwent sedation for liver

and spleen FNA. The preoperative ultrasonographic measurements of the splenic mass showed diameters ranging from 1 to 30 cm.

Due to the rupture of the splenic mass, 30 dogs (55.5%) had peritoneal effusion and 90% of patients with a histological diagnosis of hemangiosarcoma had hemoabdomen. There was a statistically significant difference in survival between dogs that presented with or without hemoabdomen, regardless of histologic diagnosis (**Figure 23**).

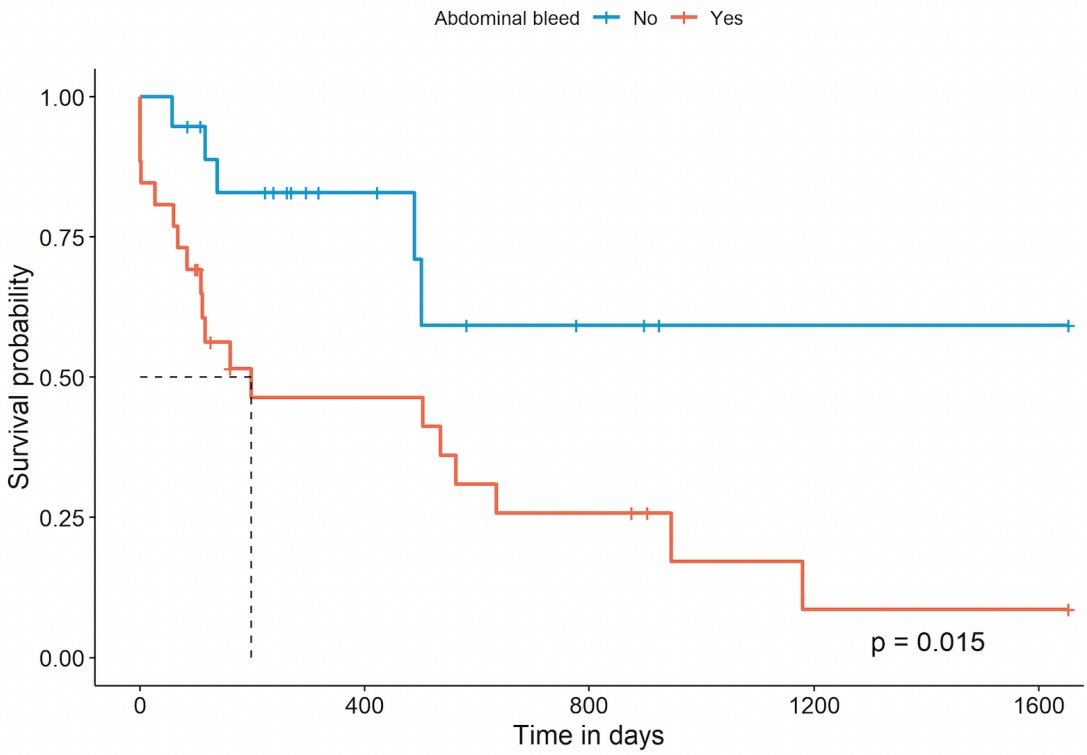

Figure 23 - *Kaplan-Meier survival curves based on the presence/absence of hemoabdomen. A significant difference (p = 0.015) between groups is evident.*

The combined effect of presence of abdominal bleeding and type of tumor (hemangiosarcoma vs other histologic diagnoses) highlighted significant differences in terms of survival probability ($p < 0.001$) (**Figure 24**).

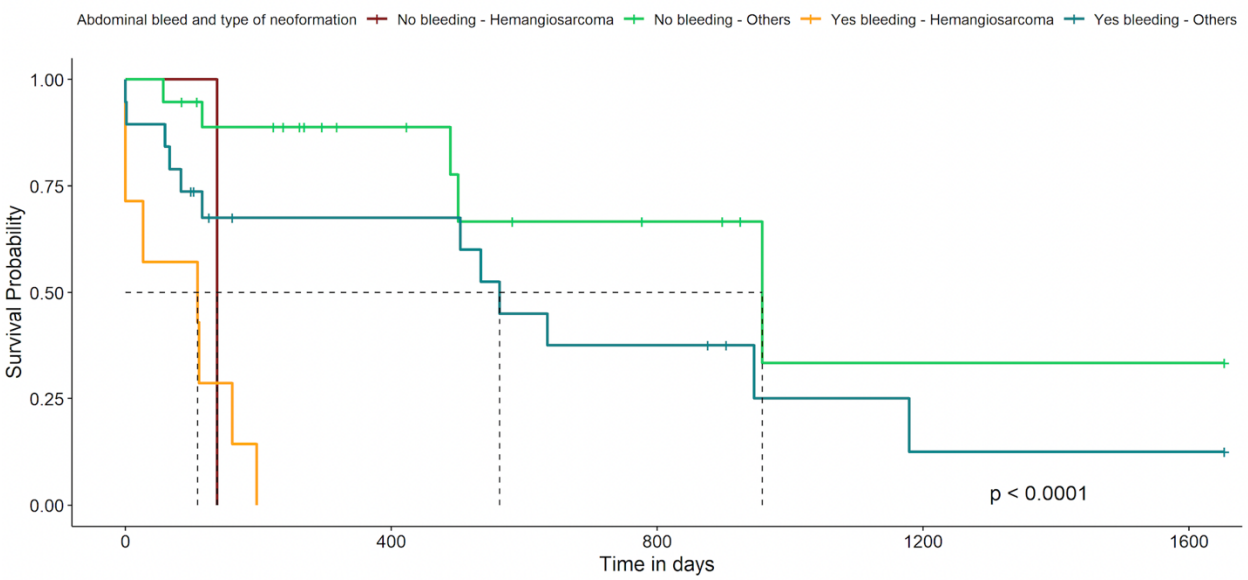

Figure 24 – *Kaplan-Meier survival curve based on combined effect of hemoabdomen and type of splenic mass.*

The comparison between benign and malignant splenic mass with concomitant hemoabdomen highlighted significant differences (p = 0.0024) in terms of survival probability with over 2x median survival probability in the case of benign tumors compared to malignant ones (**Figure 25**).

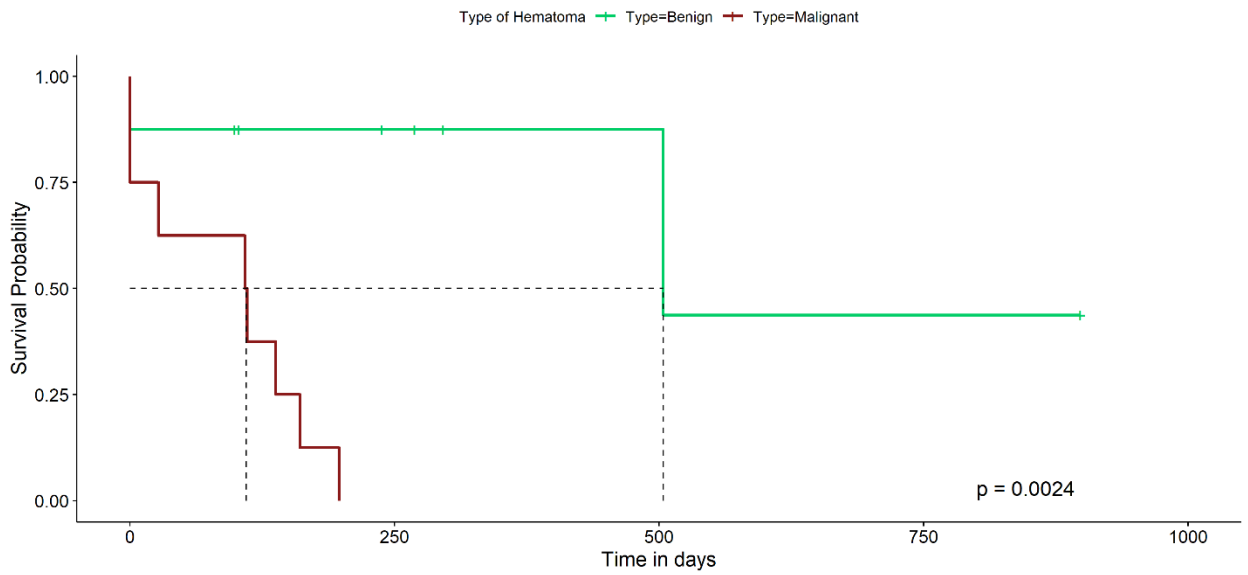

Figure 25 – *Keplan-Meier survival curve based on combined effect of hemoabdomen and benignity or malignancy of splenic mass*.

The survival probability did not differ when comparing hemangiosarcoma with other types of malignant tumors (p = 0.61) in the group of dogs with abdominal bleeding (**Figure 26**). However,

these results should be interpreted with caution due to the limited sample size of the analyzed groups.

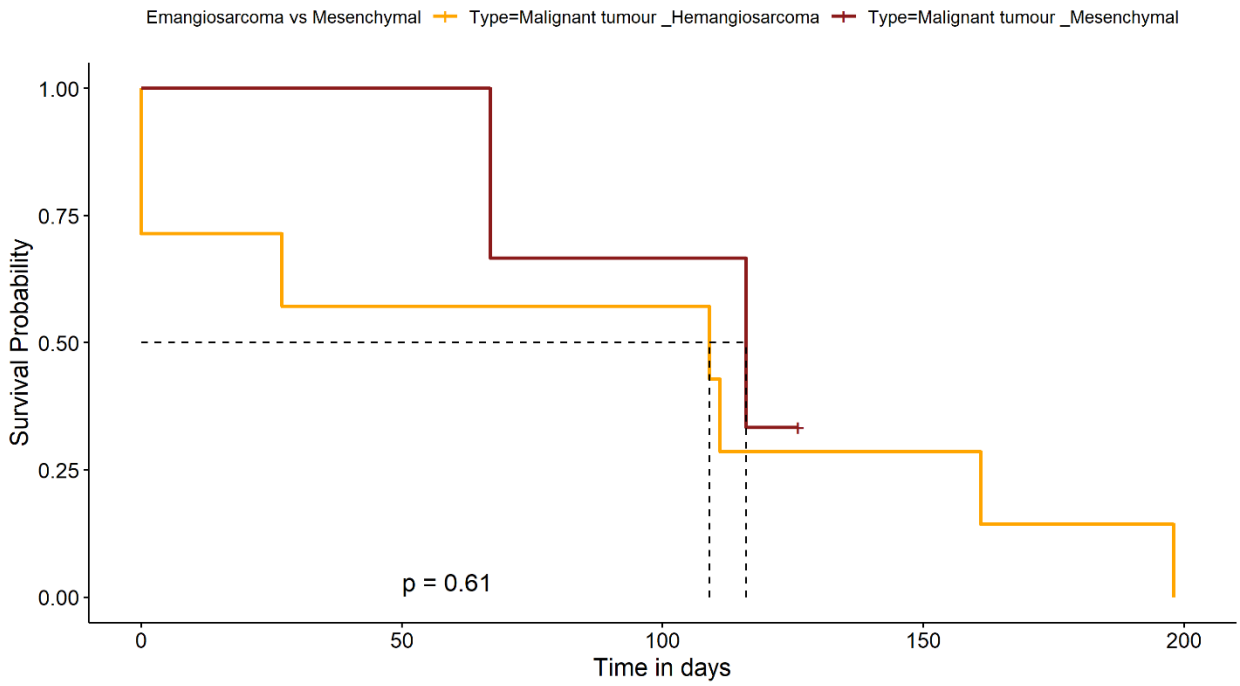

Figure 26 – *Keplan Meier survival curve based on combined effect of hemoabdomen and hemangiosarcoma or other types of malignant tumors*.

Only 3 patients (6%) required blood transfusions, either autotransfusion (n=1) or from a donor.

Five dogs (10.8%) developed intraoperative complications due to patient instability. In particular, 4 dogs were hypotensive during surgery, and 1 developed DIC, with consequent death.

A preventive gastropexy was performed in 5 large breed dogs (9.2%) during splenectomy.

According to the histological diagnoses, 17 dogs (31.5%) had splenic malignant tumors; in particular, 10 dogs (18.5% of all dogs and 58.8% of dogs with malignant tumor) were diagnosed with splenic hemangiosarcoma, 5 dogs with a tumor of mesenchymal origin (fibrosarcoma, myxosarcoma, malignant fibrous histiocytoma, perineural splenic sarcoma and undifferentiated sarcoma) and 2 dogs presented lymphoma (**Figure 27**).

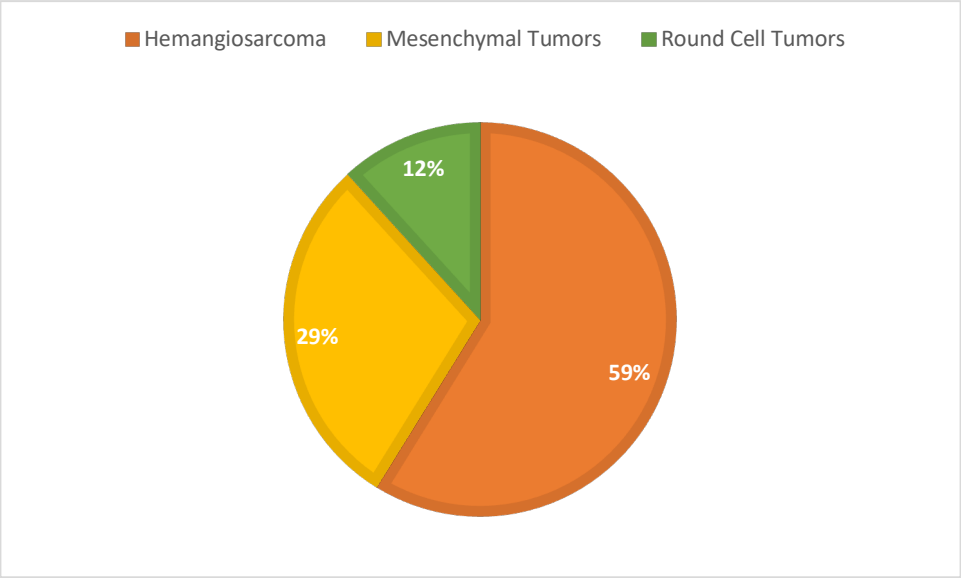

Figure 27 – *Pie chart representing different types of splenic malignant neoplasms.*

The remaining 37 dogs (68.5%) were diagnosed with non-malignancy; among these, 13 dogs (24%) had benign tumor growths, the other 24 had benign nonneoplastic diseases (**Figure 28**). In particular, the first category included:

- fibrohistiocytic nodules (n = 8);
- myelolipoma (n = 4);
- hemangioma (n = 1).

Among the benign non-cancerous diseases, there were:

- follicular hyperplasia (n = 11);
- hematoma (n = 8);
- passive congestion (n = 2);
- splenic abscess (n = 1);
- myelopoiesis (n = 1);
- pyogranulomatous splenitis (n = 1).

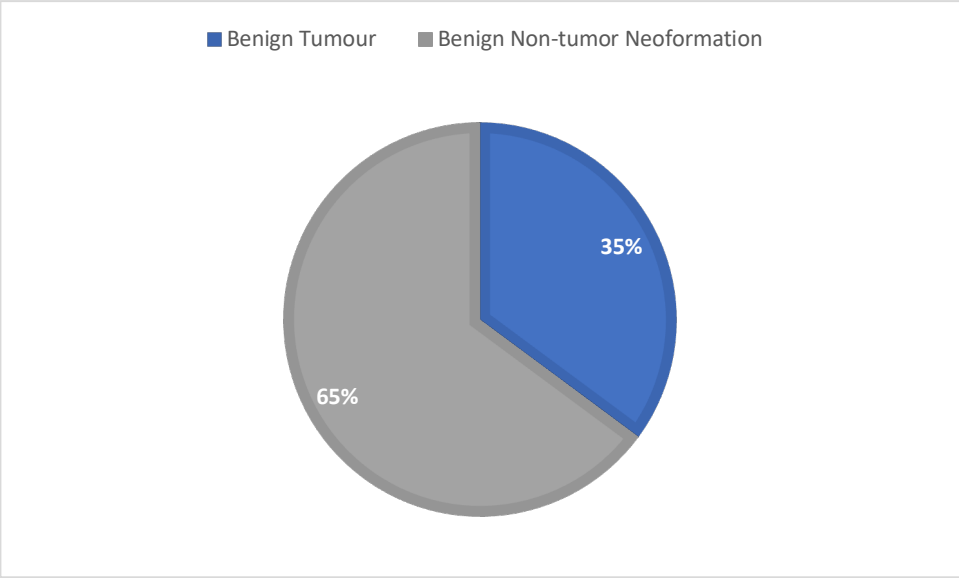

Figure 28 – *Pie chart of macro-categories of benign diagnoses*.

In addition to the histological diagnosis, in 5 dogs (9.2%) out of 54 an immunohistochemical investigation was performed, in order to have a better characterization of the tumor; in oncology, immunohistochemistry has emerged as a useful tool for identifying molecules that have therapeutic, prognostic and diagnostic value, *tumor markers*:

- 2 splenic hemangiosarcomas presented positive neoplastic cells for the endothelial marker CD31; immunohistochemistry was performed in order to differentiate them from splenic hematomas;
- 1 malignant fibrous histiocytoma presented positive neoplastic cells for the endothelial marker CD11 and negative neoplastic cells for CD31; immunohistochemistry was required in order to direct the diagnosis of pleomorphic sarcoma grade III with giant cells towards a specific histiocytic neoplasm;
- 1 perineural splenic sarcoma presented positive neoplastic cells for the GFAP + marker and negative neoplastic cells for IBA1, CD31, SMA and NSE markers; in this case, immunohistochemistry was performed in order to further investigate the primary diagnosis of splenic stromal sarcoma;
- 1 splenic follicular lymphoma had positive neoplastic cells for CD20 and CD79α; immunohistochemical investigation was performed for cluster of lineage B differentiation.

Overall survival time of the 51 dogs undergone splenectomy (excluding 3 dogs that did not satisfy the aforementioned inclusion criteria) was 223 days; in particular, it was 495 days for the 34 dogs with a benign tumor (range 1 to 2071 days) and 116 days for the 17 dogs with a malignant splenic

neoplasia (range 1 to 1023 days). Among 34 dogs with a splenic benign mass, 17 are still alive, 13 are dead for causes not related to the spleen disease, with a *MST* of 504 days and 4 were lost to follow-up after a median of 1345 days from surgery. Among 17 dogs with a malignant neoplasia, only 1 dog out of the 16 with a complete follow-up is still alive after 230 days from surgery. This dog (number 50) was diagnosed with an undifferentiated splenic sarcoma and doxorubicin-based chemotherapy regimen was administered (**Figure 29**).

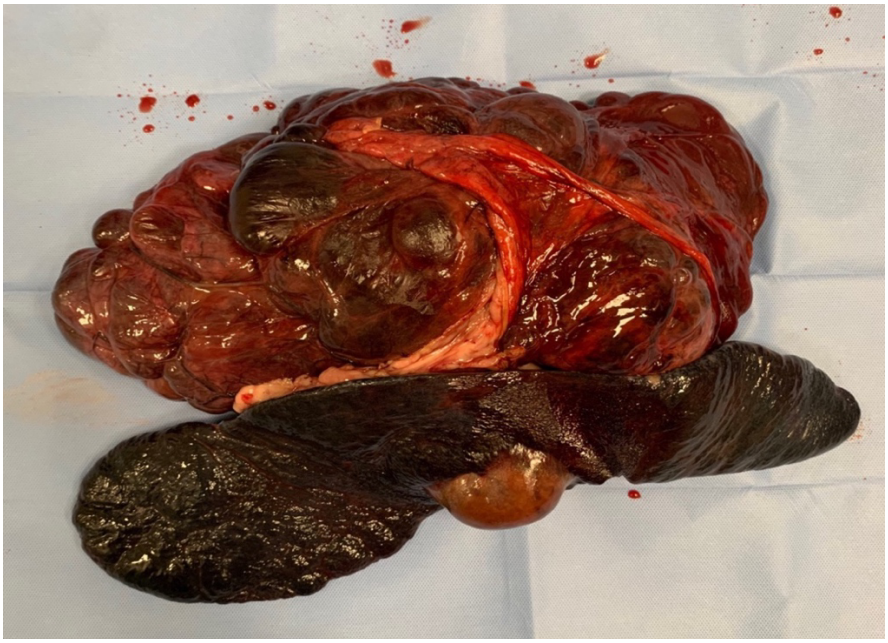

Figure 29 –*Undifferentiated splenic sarcoma. Surgery was performed at the Veterinary Teaching Hospital of the University of Parma.*

Anyway, 50% of the dogs undergone splenectomy, regardless of the underlying disease, survived for more than one year. (**Figure 30).**

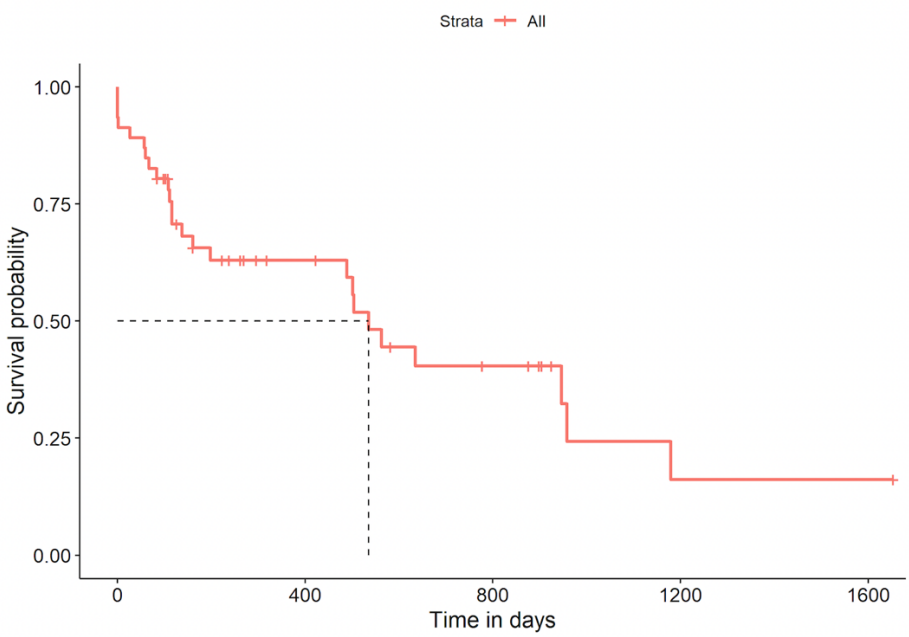

Figure 30 – *Kaplan-Meier survival curve of the whole population.*

Focusing specifically on splenic hemangiosarcoma, ten dogs (18.5%) were diagnosed with this splenic malignant neoplasia; 3 were males, 3 females, 3 spayed females and 1 neutered dog. The median age was 9 years (range 7 to 15 years). The median weight +/- SD was 34.5 +/- 13.80 kg. The most represented breed was the German shepherd (n = 3), followed by the Czechoslovakian wolf (n = 2), schnauzer, beagle, corso, boxer and dachshund (n = 1).

Three dogs had concomitant diseases:

- prosencephalic neurological problems (n=1);
- atrial fibrillation (n=1);
- chronic enteropathy (n=1).

At the time of surgery, one dog had concomitant hepatic and pulmonary metastasis.

According to the *Clinical Staging System for Canine Hemangiosarcoma* by Withrow et al. (2013), our dogs were all in stage 3.

In the current study, the size of 2/10 hemangiosarcoma was not reported; only one dog presented a small tumor (< 5 cm) while the remaining 7 subjects had a mass with a diameter between 5 cm and 10 cm.

All dogs with splenic hemangiosarcoma were dead by the end of the study and the *MST* was 124.5 days (range 1 to 1023 days). If the 2 dogs that survived more 300 days were excluded, the *MST*

was 109 days. Three out of 10 patients already had metastases at the time of surgery, with a *MST* of 27 days, and for other 3 dogs the date of metastasis detection was not reported.

When comparing the survival curve of dogs with hemangiosarcoma to those of all other splenic diagnoses (**Figure 31**), a highly significant difference ($p < 0.0001$) was observed. No dogs except 2 diagnosed with an hemangiosarcoma survived more than 198 days.

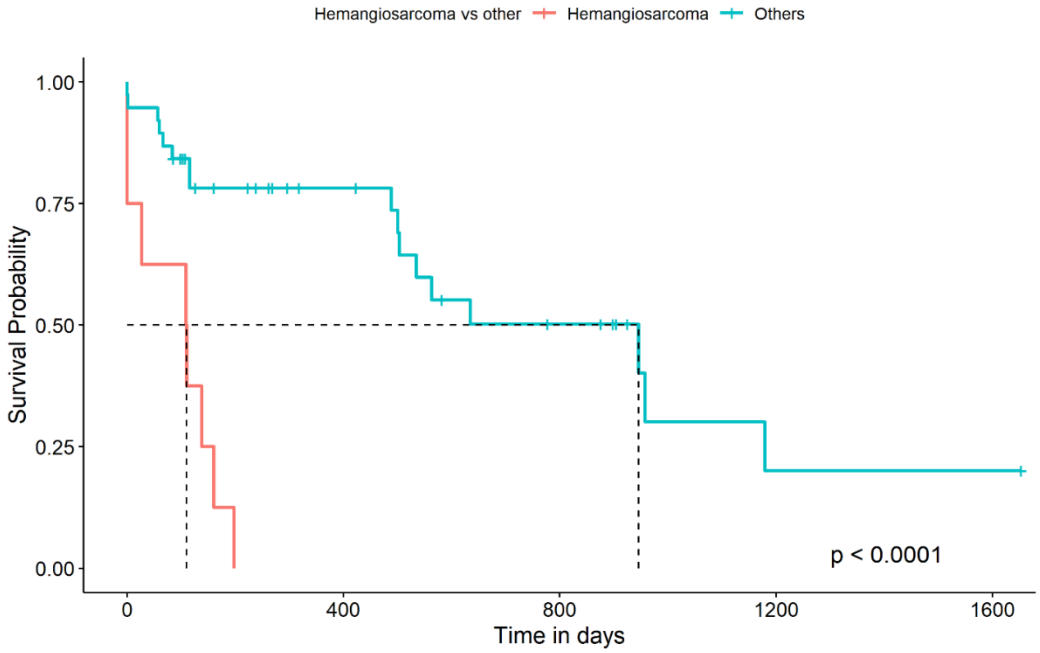

Figure 31 – *Kaplan-Meier survival curve comparing hemangiosarcoma to all other splenic diseases*.

7. DISCUSSION

Of the 54 dogs included, 30 (55.6%) and 24 (44.4%) were females and males, respectively. When considering only the 10 dogs with hemangiosarcoma, 6 were females and 4 were males. This value is not in line with what reported by several authors, according to which there seems to be a male predisposition in the development of this tumor (Gamlem, et al., 2008; Schultheiss, 2004; Brown, et al., 1985; Withrow, et al., 2013).

The median age of dogs with a histological diagnosis of splenic hemangiosarcoma was 9 years. This value is in line with the literature which reports that this neoplasm is more prevalent in adult or elderly dogs (Schultheiss, 2004; Brown, et al., 1985; Prymak, et al., 1988), aged between 8 and 13 years (Marconato, et al., 2012). No hemangiosarcoma patients were younger than 7 years old.

In our study, the overall median body weight was 25 kg, whereas the median weight for dogs with hemangiosarcoma was 34 kg. This result is compatible with the statistical data reported in the literature, according to which medium-large-sized individuals are more likely to develop hemangiosarcoma (Spangler & Kass, 1997; Story, et al., 2020; Withrow, et al., 2013).

The majority of the 54 dogs included in this study were mixed-breed (31.5%), followed by German Shepherd (11.1%), boxer (7.4%) and Labrador retrievers (7.4%).
Because of their numerical limitations, these data cannot be considered as indicative of the nationwide epidemiology. The fact that mixed-breed dogs are the prevalent breed in this study can be related to the fact that they are the most common dog breed in Italy.
Three German Shepherd dogs, two Czechoslovakian wolves, one schnauzer, one beagle, one Corso, one boxer, and one dachshund are the main breeds of the 10 dogs that received the definitive diagnosis of splenic hemangiosarcoma. According to several authors (Prymak et al., 1988; Schultheiss, 2004; Tamburini et al., 2009; Marconato et al., 2012), the German Shepherd, Labrador and Golden retriever are the most affected breeds by hemangiosarcoma.

Sixteen among 37 benign masses had a diameter larger than 10 cm, 12 had a diameter between 5 and 10 cm and the remaining 9 benign masses had a diameter smaller than 5 cm. The 70% of the 10 hemangiosarcomas had a diameter between 5 and 10 cm. This finding agrees with the literature, which indicates that splenic benign lesions can reach considerable size without causing clinical signs until rupture of the mass or compression of other organs; on the other hand,

hemangiosarcomas, despite their small size, can cause acute symptoms including hemoabdomen, collapse and sudden death due to their more friable structure (Mallinckrodt & Gottfried, 2011).

Eight of the 54 splenic masses included in this study, were incidental findings; none of them was a hemangiosarcoma. This conclusion is not unexpected, since hemangiosarcoma is characterized by rapid growth and spread, with potential early rupture.

Thirty dogs (55.5%) in total and 9 out of 10 patients with a final histological diagnosis of hemangiosarcoma presented with abdominal effusion. This finding is moderately worse compared to the literature, according to which the prevalence of HSA in dogs with non-traumatic hemoabdomen is 63-76% (Schick, et al., 2019; Hammond & Pesillo-Crosby, 2008; Burgess, et al., 2021). The prevalence of 90% in the current study may be explained by the small number of animals with hemangiosarcoma that were accounted for our study. Nontraumatic hemoabdomen, instead, is often caused by the rupture of a splenic mass or nodule, although other conditions such as coagulopathy, *GDV*, splenic torsion, liver lobe torsion and anaphylaxis have also been described as possible causes; in our study, a prevalence of almost 60% could be explained by the huge size that benign splenic neoformation could develop, with consequent possible rupture.

From our statistical analysis, which reveals that survival curves based on the presence or absence of hemoabdomen show significant differences ($p.= 0.022$), hemoperitoneum emerges as a negative prognostic factor on survival of the animals. Since the comparison between benign and malignant tumor with concomitant hemoabdomen highlighted significant differences in terms of survival probability, with over 2x median survival probability in the case of benign tumors compared to malignant ones, hemoabdomen without other malignant characteristics should not be considered a valid reason for not performing surgery.

According to the literature, the so-called "*two-thirds rule*" applies: roughly two-thirds of splenic masses are malignant and two-thirds of these are splenic hemangiosarcoma; the remaining one-third of splenic neoformations are likely benign in nature (Griffin, et al., 2021; Davies & Taylor, 2020; Withrow, et al., 2013; Gamlem, et al., 2008). The *Chi-square test* for goodness of fit was used to compare the observed distribution to the expected distribution based on literature (Spangler et al., 1997). The observed distribution in terms of number of neoplastic cases was significantly different from what expected based on the literature ($p < 0.01$), as well as the proportion of hemangiosarcoma within neoplastic cases ($p < 0.01$); in fact, in the current investigation, only 10

of the 54 dogs with splenic masses had a final histological diagnosis of splenic hemangiosarcoma and this represents a prevalence of 18.5%. Seven dogs (12.9% of the total) had a malignant splenic mass not diagnosed as hemangiosarcoma while the remaining 37 dogs (68.5%) had a benign splenic neoformation. In contrast with what is reported in the literature, this data show that 2/3 of the evaluated splenic tumors were benign, and the remaining 1/3 was malignant. Among this third, almost 2/3 were hemangiosarcoma. The information about splenic hemangiosarcoma, which accounts for 58.8% of splenic neoplasms, agrees with the literature, although it is a little lower than the average reported in several studies. This could be explained by the difference between the samples used in the study conducted by Spangler et al. (1997), which included 500 spleens, and our collection, which includes only 54 dogs. It might be interesting to check the applicability of this rule on a much larger sample.

In this study, 5 (9.2%) of 54 dogs had preventive gastropexy. To prevent *GDV*, a gastrointestinal emergency deemed potentially lethal (Sartor, et al., 2013), some studies recommend preventative gastropexy following splenectomy (evaluating it on a case-by-case basis) (Maki, et al., 2017). This low gastropexy number may be due to a variety of factors, such as patient instability, or the concurrent presence of metastases at the time of surgery, which may have judged this procedure not useful based of the short life expectancy of the dog.

Three of ten dogs with splenic hemangiosarcoma had concomitant liver or lung metastases at the time of surgery, detected on ultrasonography or chest radiography and had a *MST* of 27 days. This result agrees with the literature, according to which visceral forms of HSA are highly metastatic (Masyr, et al., 2021).

Among dogs arriving at the Veterinary Teaching Hospital without hemoabdomen, only 4 underwent sedation to perform splenic and hepatic FNA prior to surgery. According to a study conducted by Tecilla et al. (2019), cytology may be a helpful diagnostic method, necessitating surgery independently of other diagnostic procedures, when it is diagnostic for neoplasia.
On the other hand, a nondiagnostic cytology result requires further investigation to confirm that the dog does not have a splenic pathology. Although histopathology is generally considered the diagnostic gold-standard (Ballegeer, et al., 2007), several limitations for this diagnostic technique should also be considered in case of splenic tumors. In particular, splenic hematomas and hemangiosarcomas are thought to be challenging to differentiate, especially if the spleen is not fully analyzed and if adequate samples are sent to the pathologist (Spangler & Kass, 1997; Eberle,

et al., 2012). Furthermore, splenic hematomas and hemangiosarcoma may not be grossly distinguishable (Christensen, et al., 2009) and the first one could represent a component of the latter (Patten, et al., 2016).

The absence of post-splenectomy chemotherapy treatments in all hemangiosarcoma cases included in this study is an unexpected discovery. According to the literature, dogs with stage III HSA which underwent surgery alone, have a *MST* from 27 to 65 days, as opposed to dogs that received also an adjuvant chemotherapy at maximum tolerated dose (*MTD*), including an anthracycline as a single agent, doxorubicin in combination with cyclophosphamide or dacarbazine, or doxorubicin in combination with vincristine and cyclophosphamide, and reached a *MST* from 62 to 195 days (Marconato, et al., 2019). Alternative treatments such as metronomic chemotherapy and immunotherapies were attempted in the same study, but they did not improve survival times. Another study evaluated carboplatin as a potential substitute for doxorubicin (*MST* of 160 days), particularly in dogs predisposed to the cardiotoxicity caused by doxorubicin (Faulhaber, et al., 2021). Carboplatin can also be used in dogs with *abcb1* mutations as it is not a substrate for P-glycoprotein (Mealey & Fidel, 2015).
The lack of a medical oncology department in our Veterinary Teaching Hospital (which opened only in 2021) or the owners reluctance to follow a chemotherapy protocol are two possible explanations for the absence of post-operative chemotherapy in dogs having splenectomy surgery. The *MST* of our group of hemangiosarcoma dogs, however, was 109 days without any chemotherapy protocol, which is comparable to the information provided above.

Patients who underwent splenectomy and whose histologic diagnosis was hemangiosarcoma, had a *MST* of 124.5 days and 109 days excluding two outliers. This result agrees with those found in the literature, which suggests that *MST* for a stage I splenic hemangiosarcoma (limited to the spleen) is approximately 239–355 days, compared to a *MST* of 120-148 for a stage II hemangiosarcoma (ruptured or involving the regional lymph node) (Vail, et al., 1995) or a *MST* of 23-40 days for a stage III hemangiosarcoma (Lucroy, et al., 2020). In our study, all cases of hemangiosarcoma were stage III, and 9 out of 10 patients had hemoabdomen at the time of diagnosis.

The 2 long surviving dogs with hemangiosarcoma would probably require a second histological analysis, considering the *MST*s that have been documented in the literature. Patient *number 17*, a 7-year-old beagle who underwent surgery and reported a ST of 1023 days and patient *number 20*,

an 8-year-old boxer, underwent surgery for a ruptured hemangiosarcoma and reported a ST of almost 600 days. Given the difficulties of interpretation of histology specimens and the fact that all of them significantly surpass the *MST* reported in the literature, it would be appropriate to perform additional histological and immunohistochemical evaluations. For the mentioned reasons, their STs were not deemed statistically evaluable.

This study has some limitations that must be considered during the interpretation of the results. First of all, the retrospective nature and interfacing only with owners can determine inaccuracy of some information, especially regarding follow-up. Second, only 54 patients were included, which may have had an impact on the statistical results. In addition, some patients were excluded due to lack of valid follow-up or due to the lack of a pathological diagnostic report of the removed spleen. A further limitation is attributed to different veterinary laboratories which performed the analysis, with consequent potential error.

8. CONCLUSION

The study evaluated the main characteristics of dogs undergoing splenectomy at the Veterinary Teaching Hospital of the University of Parma, with particular focus on those with a histological diagnosis of splenic hemangiosarcoma.

Information generated from breeds involved, body weight, age of onset and size were comparable to what is reported by several authors in the literature and can therefore be considered a further confirmation.

Overall survival time of 51 dogs that underwent splenectomy (excluding 3 dogs that didn't satisfy the aforementioned inclusion criteria) was 223 days; it was 495 days for the 34 dogs with a benign tumor (range 1 to 2071 days) and 116 days for the 17 dogs with a malignant splenic neoplasia (range 1 to 1023 days). The *MST* for 10 dogs that were diagnosed with splenic hemangiosarcoma was 124.5 days (109 days without two outliers), and all of them have died for tumor progression.

More animals compared to what is reported by the literature presented hemoabdomen at the time of diagnosis and, above all, "*the 2/3 rule*" could not be confirmed in this study because only one-third of the masses were malignant.

In light of the results of this study, which revealed that splenic masses and hemoabdomen in older dogs do not necessarily have malignant significance, since hemoperitoneum was detected even in case of benign neoformations rupture, surgery should be performed in these cases without any hesitation.

This study therefore represents a preliminary analysis with the expectation of a potential future prospective study involving a larger number of dogs and the execution of periodic post-operative checks to avoid inaccuracies resulting from the interface with owners. Furthermore, given the frequency of hemangiosarcoma in predisposed breeds, it would be interesting to exploit preventive medicine by having six-monthly ultrasounds check to correctly identify the precise age of onset and determine prognosis and survival times in patients where these diseases are mainly diagnosed, in absence of metastases.

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