

Systems Pathology: Hemolymphatic

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About

These notes are a fairly comprehensive collection of information to complement the lectures and labs delivered in Systems Pathology, on the topic of the hemolymphatic system. Although all of the information is useful, certain areas will have been emphasized in lecture. Let the areas focussed on in lectures and lab guide your studying. There are a few rare conditions that are not discussed in these notes, and you are encouraged to read through the relevant chapters in the textbooks recommended below.

The notes are available online at <http://russfraser.ca/hemolymphatic/>, as a PDF on Moodle, and as an Epub (E-book format, suitable for a tablet or e-reader). Please feel free to provide feedback, whether on content, style, or typos!

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Please don't hesitate to get in touch if you have any questions.

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Reference material

- Zachary, J. F., & McGavin, M. D. (2016). *Pathologic Basis of Veterinary Disease Expert Consult*. Elsevier Health Sciences.
- Maxie, G. (2015). *Jubb, Kennedy & Palmer's Pathology of Domestic Animals-E-Book* (Vol. 3). Elsevier Health Sciences.

Acknowledgements

These lecture notes were prepared in R (R Core Team, 2019) using the bookdown (Xie, 2015), knitr (Xie, 2019), and Rmarkdown (Allaire et al., 2019) packages. Source material was taken from Zachary and McGavin (2016) and Maxie (2015). I gratefully acknowledge the prior course notes from Dr. Shannon Martinson. Images are attributed throughout the text; unattributed images are either mine or were found in the public domain.

1 Introduction

The hemolymphatic system is composed of the hematopoietic system, cells within the circulation, and the lymphoid system. The hematopoietic system is largely restricted to the bone marrow, while the cells of the circulation are found not only in the blood, but often migrate into tissues. The lymphoid system is widely distributed and is part of several organs, including the lymph nodes, spleen, and various distributed “associated lymphoid tissues” (ALTs), such as the mucosal associated lymphoid tissue (MALT), bronchiolar associated lymphoid tissues (BALT), etc., etc. Regardless of the location of the lymphoid tissue, it’s normal immunological reaction is the same.

The separation of topics in this section is a bit artificial, and could easily be organized differently. The presentation here is the way in which it makes most sense to me, but you may find the organization in the reference textbook more to your liking. The information is the same.

2 Bone marrow

2.1 Introduction

Bone marrow is found within the spongy portions of bones. It is the site of production of the cellular elements of blood, known as hematopoiesis, which can be broadly divided into **myeloid** and **lymphoid** components. The lymphoid lineage produces lymphocytes, while the myeloid lineage produces everything else (Fig 1). The massive number of cells produced by the bone marrow all originate from a common stem cell, which has the potential to differentiate into any type of cell type found in the blood. The first division commits a stem cell either to the *lymphoid* or *myeloid* lineage. Under the influence of growth factors and cytokines, they gradually differentiate as they divide, eventually becoming a fully differentiated, functional leukocyte, erythrocyte, or thrombocyte. Early stages of differentiation are morphologically indistinguishable, and the cells at this stage are often referred to as “blast” cells, short for the various names of immature cells (e.g. monoblast, myeloblast, rubriblast, etc.). The bone marrow is composed of cells of a variety of maturational stages, but **mature, morphologically identifiable cells outnumber immature “blast” cells significantly.**

The location of hematopoiesis changes as an animal grows. *In utero*, the primary sites of hematopoiesis are the liver and spleen. In neonatal and young animals, the marrow spaces of virtually all bones is occupied and generating cells. In adults, hematopoietic tissue is mostly restricted to axial bones (i.e. skull, vertebrae, ribs, sternum, and pelvis), as well as the proximal portion of the humerus and femur. As the hematopoietic tissue recedes during aging, it is replaced by fat.

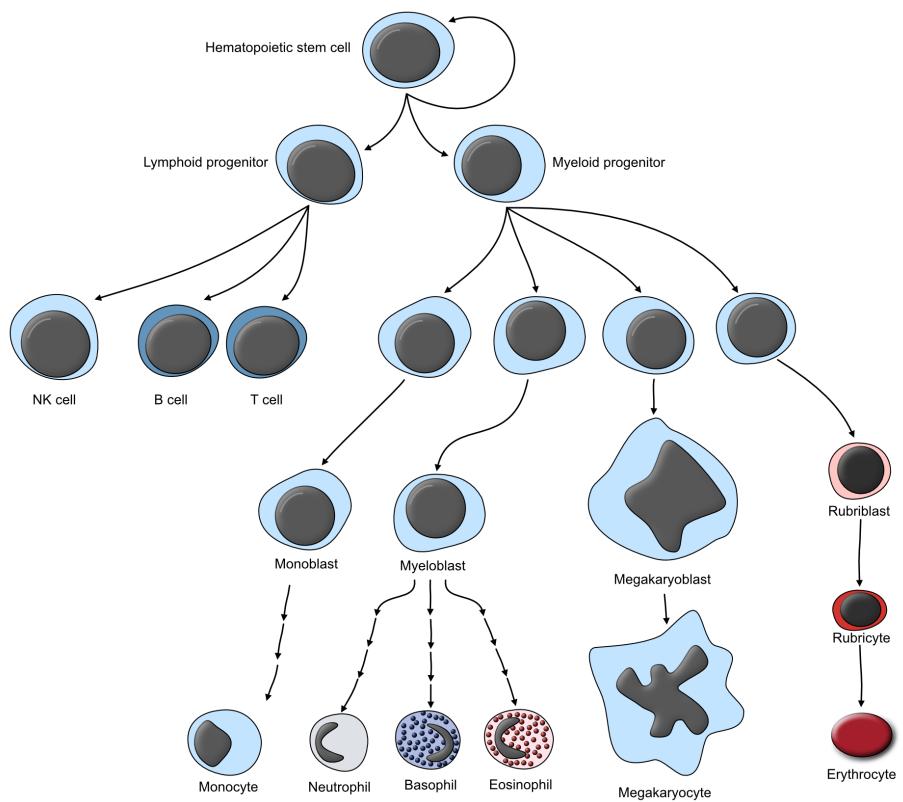


Figure 1: Schematic illustrating the progressive maturation of the various lineages of circulating blood cells.

Because the products of hematopoiesis are easily evaluated by sampling the blood, biopsy or aspirate of the bone marrow is relatively uncommon. Similarly, because evaluation of the blood can reveal quite a bit about the underlying bone marrow, *many of the diseases of bone marrow are better evaluated by clinical pathologists.*

2.2 Adaptations of growth

2.2.1 Hyperplasia

Hyperplastic bone marrow is a common finding. **The bone marrow responds to abnormally low levels of circulating cells by ramping up production of that particular cell type.** Thus, many causes of anemia, inflammation, or thrombocytopenia can lead to marrow hyperplasia. Occasionally, the marrow cannot produce the required cells. For example, the production of red blood cells (RBCs) requires iron-containing hemoglobin. In patients that are iron deficient, producing new RBCs is not possible, and thus hyperplasia will not occur.

Grossly, the typically fatty portion of the marrow (i.e. the diaphysis of the long bones) changes from yellow to red. **Hyperplasia of ANY cell line, not just the erythroid cell line, turns the marrow red.**

2.2.2 Hypoplasia

Bone marrow hypoplasia refers to the decrease in or absence of production in one or more cell lines. Causes are numerous and varied. Lack of signals for growth and differentiation can lead to hypoplasia. For example, erythropoietin (EPO) stimulates differentiation and production of RBCs from the marrow. EPO is produced by the kidney. In cases of chronic renal disease, EPO production can decrease, leading to a concurrent decrease in the cells of the erythroid lineage (i.e. erythroid hypoplasia). Lack of nutrients can lead to hypoplasia of one or more cell lines. To continue the example from the section on hyperplasia, an absence of iron leads to an inability of the erythroid lineage to mature, and can result in hypoplasia. Degeneration and necrosis, which itself has a number of causes, can lead to bone marrow hypoplasia.

Grossly, hypoplastic bone marrow is reflected by an increase in the pale yellow component of the marrow and decrease in the red portion.

2.2.3 Serous atrophy

Serous atrophy of fat refers to the transformation of the normal fat component of the bone marrow into a gelatinous, translucent material. **It is common,**

and usually associated with malnutrition or cachexia. It is a non-specific indicator that the animal is chronically ill.

2.3 Degeneration and necrosis

Degeneration and necrosis of the bone marrow, depending on the extent, can have significant consequences for the animal. The most common presenting sign is a cytopenia in one or more cell lines. Recall that hematopoietic cells are metabolically and mitotically active: this renders them susceptible to a wide variety of insults. However, so long as the target of damage is not the hematopoietic stem cell (Fig 1), it is likely that the bone marrow will be capable of recovering, so long as the insult is removed.

Common causes of bone marrow damage include:

1. Damage from radiation
2. Infectious agents
 - Feline immunodeficiency virus, feline leukemia virus, feline pan-leukopenia
 - Equine infectious anemia virus
 - Canine parvovirus 2
3. Immune-mediated diseases
4. Idiopathic
5. Toxins and drugs
 - Certain chemotherapeutic agents
 - Idiosyncratic drug reactions
 - Toxic substances

2.4 Neoplasia

Before diving into this section, some explanation is needed. What is going to be discussed here are neoplasms that arise primarily from the precursor cells within the bone marrow itself, namely leukemias. A broader classification system uses the umbrella term “hematopoietic neoplasia” to define neoplasms that arise from any of the formed elements of blood. Thus, for example, a cutaneous mast cell tumour would be considered a hematopoietic neoplasm, because it is caused by a cell originally from the bone marrow, even though it forms a tumour in the skin. **These types of tumours will not be discussed here, but will be discussed in other sections.** A list of tumours *not* discussed here includes:

- Mast cell tumours (skin or GI)
- Plasmacytomas (skin)
- Cutaneous histiocytomas (skin)

Lymphoma is a very important disease, and is discussed in the section on lymph nodes (though it could arguably be included here.)

Broadly, neoplasms of the marrow are separated based on their lineage of origin. Neoplasms of lymphoid origin include lymphoid leukemias, lymphoma, and plasma cell tumours. Neoplasms of the myeloid lineage include myeloid leukemias, myelodysplastic syndrome, histiocytic tumours, and mast cell tumours.

2.4.1 Myeloid neoplasms

2.4.1.1 Acute myeloid leukemia (AML)

AML is defined as an acute cytopenia accompanied by > 20 % blast cells in either the blood or bone marrow (or both). Recall that blast cells are immature cells that cannot be reliably identified by cytology or histopathology (in other words, it is a precursor cell that has not differentiated enough to have recognizable features). Cytopathologists are best suited to evaluate the number of blast cells, while histopathology of the bone marrow is useful to characterize the extent of myelophthisis (replacement of normal marrow elements by other tissues), and/or necrosis and fibrosis. In an ideal world, the type of AML could be further categorized by looking for specific differentiating markers on the neoplastic blast cells; in reality, the cost is still high, and utility of doing so is debatable.

Animals with AML typically present with significant acute, non-specific illness. Routine bloodwork often reveals cytopenia of one or more cell line that cannot be explained. Peripheral blood may or may not contain blast cells, in which case evaluation of the bone marrow by cytology or histopathology needs to be pursued. The liver and/or spleen may be diffusely enlarged in some cases. The prognosis for animals with AML is very poor.

2.4.1.2 Myelodysplastic syndrome

Myelodysplastic syndrome present primarily in cats and dogs as an acute, non-specific illness. Bloodwork demonstrates one or more non-regenerative cytopenias and dysplasia of cells either in the blood or bone marrow. The bone marrow contains an increased proportion of blast cells, between 5 - 20 %. A variety of sub classifications exist. Non-regenerative anemia is frequently present. **In dogs, MDS often precedes AML.**

2.4.1.3 Myeloproliferative neoplasms (chronic leukemia)

This is a very rare condition in animals, and is characterized by the slow accumulation of very large numbers of well-differentiated cells of the myeloid lineage. It is typically an incidental finding on routine bloodwork in older animals. Very large numbers of erythrocytes, thrombocytes, or various leukocytes may be

noted in the blood. Other peripheral cytopenias are rare, and the bone marrow is not necessarily affected.

2.4.2 Lymphoid neoplasms

2.4.2.1 Acute lymphoid leukemia (ALL)

ALL is the lymphoid equivalent to AML. It is characterized by > 20 % of lymphoid blasts in the circulation and/or bone marrow. One or more cytopenias are usually noted on CBC, and there may be involvement of the liver or spleen. Feline leukemia virus is a predisposing factor in cats. In dogs, B-cell ALL is more common than T-cell. In dogs, ALL can enlarge lymph nodes, and it can be difficult to distinguish from a specific subtype of lymphoma.

Clinical signs are vague and non-specific, but are usually acute in onset. Prognosis is very poor.

2.4.2.2 Chronic lymphoid leukemia

This is the most common type of leukemia in dogs. Most are of T-cell origin. This is an indolent form of leukemia, with a slow accumulation of lymphocytes either within the bone marrow (B-cell origin CLL) or spleen (T-cell origin CLL). Eventually the disease manifests with a profound lymphocytosis. Prognosis is fair to good, with most animals living 1 - 3 years after the diagnosis.

2.4.2.3 Multiple myeloma

Multiple myeloma is a **malignant tumour of plasma cells**, usually found in older animals. Dogs, and less frequently horses and cats, are affected. Tumours usually appear within the medulla of bones with active bone marrow, frequently the vertebrae and pelvis, leading to pain, weakness, or paresis. The tumours are **osteolytic**. As you might expect from a plasma cell tumour, the neoplastic cells secrete large quantities of a clonal immunoglobulin, which can be easily detected on routine bloodwork as a hypergammaglobulinemia. With electrophoresis, it can be determined that it is a **monoclonal gammopathy**. Proteinuria is also common, as the tumour produces free immunoglobulin light chains that freely pass through the glomerulus, known as **Bence-Jones proteins**. The diagnosis of multiple myeloma requires identification of not just the neoplastic cells within the bone marrow, but also monoclonal gammopathy, Bence-Jones proteinuria, or osteolysis.

In cats, osteolysis is not common, and the tumour more often appears in the abdominal organs or skin.

2.5 Biopsy of the bone marrow

Marrow biopsy is somewhat rarely performed in daily clinical practice. There is nothing preventing you from biopsying the marrow, but it *is* difficult. You need proper equipment and technique. The relatively uncommon need for biopsy leads to clinician discomfort with a procedure they rarely perform. Along those lines, many cases with indications for bone marrow work-up end up being referred to specialty centers, where specialists end up performing the biopsies.

Work-up of the bone marrow should **always include three submissions: 1) bone marrow biopsy, 2) bone marrow aspirate, and 3) CBC from the time of biopsy.** Any historical CBCs should be also made available to the pathologists.

Biopsies are routinely taken from the humerus, femur, or ilium in small animals. In large animals, the sternum and tuber coxae are preferred sites. Aspirates and biopsies can be performed simultaneously. Bone marrow aspirates are delivered to clinical pathologists, who can best characterize and classify the aspirated cells. Core biopsies are sent to anatomic pathologists, who can identify architectural disturbances and the presence of abnormal marrow components, such as myelofibrosis, neoplasia, or inflammation.

A note on terminology: This is an “FYI” part of the notes, but may be useful to know. The terminology surrounding the evaluation of bone marrow has changed in recent years. One of the important metrics used to evaluate bone marrow is the ratio between cells of the granulocytic and macrophage lineages vs. those in the erythrocytic lineage. Historically, this was referred to as the “myeloid:erythroid” ratio (M:E ratio). This is inaccurate, however, as technically both erythroid and granulocytic cells are part of the myeloid lineage. The terminology has now shifted, such that this evaluation is reported as the “granulocytic:erythroid ratio”. If you see the older, “M:E” term in the literature, it is referring to the same thing as the “G:E” ratio. Fascinating stuff.

3 Spleen

3.1 Review

Diseases involving the spleen are relatively common in dogs, but less so in other species.

The spleen is a complex organ composed of two physical and functional spaces: the red and white pulp. It serves three main functions:

1. Filtering out aged or altered RBCs and blood-borne particulate matter.
2. Screening blood for pathogens.
3. Storage of blood (RBCs and platelets in particular)

As with other organs, reviewing the normal structure and function of the spleen will help you understand the pathogenesis of the conditions described below. Chapter 13 of Pathologic Basis of Veterinary Disease provides a good overview, and can be accessed from the library.

A few key points are worth mentioning here, however.

- The spleen is made up of red and white pulp.
- The white pulp is composed of the periarteriolar lymphoid sheath (PALS), the germinal centers, and the marginal zone.
- The red pulp is composed of the splenic cords, vascular sinuses, and macrophages, lymphocytes, plasma cells, and erythrocytes.
- There is no lymph supply to or from the spleen.

Broadly, think of the spleen as a screening and filtering organ. The blood is screened for pathogens and aged RBCs, and inappropriate organisms or material are filtered out.

3.2 Examination of the spleen

When examining the spleen, take care to note the size (enlarged vs normal vs small), the presence of nodules, any areas of discolouration, and any changes in texture. The spleen should be serially sectioned: cross-sectional slices approximately 1 cm thick made through the entire spleen from head to tail.

Small spleens are generally unimportant. Splenic aplasia is extremely rare, and unlikely to be encountered.

Large spleens (“splenomegaly”) are of greater concern. Large spleens may be diffusely enlarged, or enlarged by single or multiple nodules. On cut-section, they may be bloody (i.e. ooze blood on cut section), or ‘meaty’ (i.e. enlarged by something other than blood). Although there is overlap between these phenotypes, broadly classifying spleens in this way can help narrow down a list of differential diagnoses ((Table 1).

3.3 Circulatory disturbances

3.3.1 Congestion

With their enormous capacity to store blood, spleens can vary tremendously in size. Congestion leads to an enlarged spleen that oozes blood on cut section. The most common cause of an enlarged, congested spleen is **barbituate euthanasia**, but splenic torsion, acute hemolytic anemia, or shock can also lead to a congested spleen.

Table 1: Differential diagnoses for different types of splenomegaly (not an exhaustive list)

Features	Nodular	Diffuse
Bloody	[Hematoma], [hemangiosarcoma][Hemangiosarcoma],[acute splenic infarct][Necrosis and infarction], incomplete splenic contraction	[Congestion], splenic torsion,[septicemia][Sepsis], hemolytic anemia
Meaty	[Nodular hyperplasia], [various sarcomas][Non-angiomatous, non-lymphomatous sarcomas], metastatic neoplasia, [granulomas][Chronic splenitis]	Histiocytic sarcoma[Hemophagocytic histiocytic sarcoma], [leukemia/lymphoma][Lymphoma], amyloidosis

3.3.2 Hematoma

Hematomas are common masses found in the spleen, particularly of dogs. They can range greatly in size, from 1 - 2 cm up to very large, 20 - 30 cm masses. They are dark red, poorly demarcated from the adjacent splenic parenchyma, and are bloody on cut section. They may be caused by trauma, or by alteration of the circulatory system caused by other benign growths, such as nodular hyperplasia. Rupture of these masses can lead to hemoabdomen and hypovolemic shock. **These masses cannot be grossly differentiated from hemangiosarcoma, and thus must be submitted for histopathology.**

3.3.3 Necrosis and infarction

Infarctions are common lesions found in the spleen. Due to the nature of the vascular supply of the spleen, there is little collateral circulation, rendering them prone to infarction. Acute venous infarcts (Fig 2) generally appear bright red, well demarcated, and may bulge slightly on cut section. As the infarct matures, the necrotic tissue is removed and replaced by fibrous connective tissue (Fig 3), and becomes pale and firm (in other words, the spleen develops a scar).

Although splenic infarctions are often incidental findings, they may be key lesions in certain diseases. Infectious diseases causing vascular damage, for example classical swine fever virus or sepsis, can lead to infarction. Hypercoagulable



Figure 2: Irregularly arborizing areas of deep red are noticeable along the surface of this spleen, consistent with acute necrosis.



Figure 3: Along the left lateral aspect of this cross-section of a spleen, there is a pale white, well demarcated area consistent with a chronic infarct

states, seen with immune-mediated hemolytic anemia or as a paraneoplastic syndrome, can result in infarction. Endocarditis (bacterial infection of the heart valves) can result in septic emboli or in thrombi, which can lodge in the small vessels of the spleen and lead to infarction.

3.4 Inflammation and infection

Unlike some other tissues (for example, liver or lung), infections only rarely settle and become chronic in the spleen, perhaps due to the large numbers of phagocytic cells. The spleen, however, can react to blood-borne pathogens, or to pathogens that target lymphoid tissues. A few examples of each are discussed below.

3.4.1 Sepsis

Given its role in filtering the blood, it is understandable that the spleen would react to massive blood-borne infections of sepsis. In some instances of sepsis, the spleen is markedly enlarged by blood, soft, and dark red.

3.4.1.1 Anthrax

Anthrax is important not because it is a frequent disease (it's rare), but because it is **fatal to many animals, including humans** (it's also **reportable**). It is caused by *Bacillus anthracis*, a gram-positive, spore-forming bacteria. Herbivores in particular are susceptible, while reptiles and carnivorous birds are resistant. Infection is generally acquired through the disturbance of soil (e.g. excavation), which exposes the spores that are *highly resistant to the environment, and can live for long periods (years) in adverse conditions*. Cattle and sheep ingest spores, and spores enter circulation through traumatized mucous membranes. Carnivores may become infected by consuming the carcass of an infected herbivore, or through direct consumption of the spores. Following ingestion, the spores germinate and traffic to lymph nodes, causing a lymphangitis and lymphadenitis that then progresses to septicemia. The bacteria produce 3 key toxins: **edema factor, lethal factor, and protective antigen**. These toxins contribute to vascular damage and impaired coagulation, along with injury and inactivation of phagocytes. **Death is usually rapid (< 24 hours), especially in sheep and goats**. Cattle may also die rapidly, but some probably recover.

The classical presentation of a case of bovine anthrax is sudden death of the animal with little to no clinical signs. The animal will often **ooze blood from its orifices**, and will appear to have putrified more rapidly than expected. **Marked splenomegaly is common**, and blood fails to clots.

There are 2 reasons why you will hopefully never see the splenomegaly associated with anthrax. 1) It is a rare (and zoonotic!) disease. 2) **You should not necropsy an animal you suspect is affected with anthrax.**

To elaborate on point 2: massive replication of *B. anthracis* in the host occurs prior to death. **Upon exposure to air, the organisms sporulate, forming the highly resistant organisms that can contaminate the environment.** Thus, instead of necropsying these animals and getting anthrax spores all over the place, a blood smear taken prior to necropsy can identify the organisms. Rapid body-side test kits for *B. anthracis* are also available.

3.4.2 ASFV and CSFV

African swine fever (ASF) virus and classical swine fever (CSF) virus are two **reportable diseases** of swine. They both affect a variety of organs, and the spleen is one. Both diseases cause lymphoid depletion. CSF often leads to hemorrhagic infarcts, while ASF causes marked splenomegaly.

3.4.3 Chronic splenitis

This is relatively uncommon. Granulomas, presenting either as multifocal pale nodules or a diffusely pale spleen, can be caused by *Mycobacterium* spp. and systemic fungal diseases such as histoplasmosis or blastomycosis.

Splenic abscesses are similarly uncommon. *Rhodococcus equi* (in horses) is perhaps the most common agent, along with *Truperella pyogenes* (cattle).

3.5 Hyperplasia and neoplasia

Neoplastic diseases of the spleen present either as nodules or as a diffusely enlarged, meaty (but not bloody) spleen. The former is more common. **It is not possible to differentiate grossly between nodular lesions on the spleen,** biopsy and submitting them for histopathology is the only way to diagnose these masses.

The most important neoplasm of the spleen is hemangiosarcoma.

3.5.1 Nodular hyperplasia

Nodular hyperplasia is a common finding in the spleens of dogs, and to some degree bulls. Nodular hyperplasia simply refers to the proliferation of elements of the spleen to the extent that they form a nodule. The elements may be lymphoid in origin (lymphoid nodular hyperplasia), may be foci of extramedullary hematopoiesis, or may have a mixture of varying components. They are benign lesions, but can be difficult to grossly differentiate from hemangiosarcoma.



Figure 4: A large, red, friable, partially necrotic mass is present at the approximate midpoint of the spleen. Multiple other variably sized, red, raised masses are present randomly throughout the remaining parenchyma.

They are usually small (up to 2 cm, but can be larger), and often have irregular areas of palor on cut-section. They may be a predisposing factor for the development of a splenic hematoma.

3.5.2 Hemangiosarcoma

Hemangiosarcoma is the most common malignant neoplasm of the spleen in dogs, and is almost invariably fatal. **There is no cure**. The cause of the disease is uncertain, but likely multifactorial.

The gross appearance of hemangiosarcoma is variable (see Fig 4). The neoplasm may appear as one or multiple nodules, some of which may be ruptured. It is not uncommon for a portion of the omentum to adhere to ruptured nodules. Nodules are generally diffusely red or mottled red and white, bloody on cut section, and are poorly demarcated from the adjacent spleen. Larger nodules are often necrotic at their core.

Histologically, hemangiosarcomas are composed of thin-walled, endothelial-lined channels filled with blood. As you can imagine, these channels are fragile, and one of the most common and serious complications of hemangiosarcoma is **rupture**, leading to variably profound hemorrhage (hemoabdomen) and shock.

Hemangiosarcomas are very prone to metastasis, and frequently metastasize to the **liver and lungs** (Fig 5), as well as the heart and CNS. In the liver and

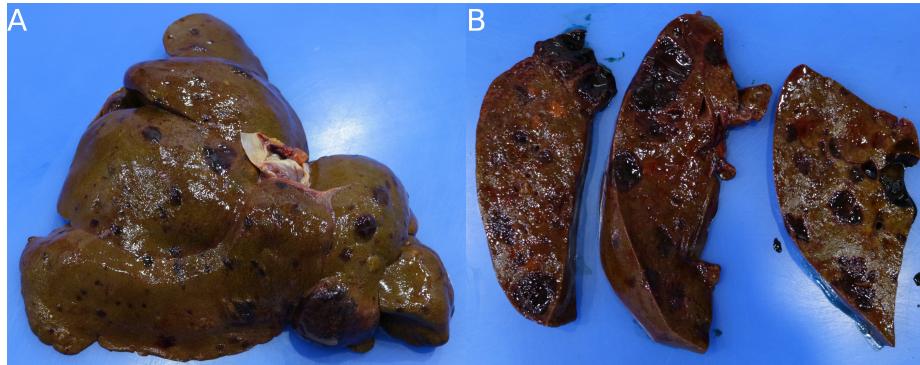


Figure 5: A) A canine liver with multiple, variably sized, raised, roughly spherical masses protruding from the capsular surface. B) On cut section, the masses are red, gelatinous, well-demarcated, and roughly spherical.

lungs, metastatic nodules are distinctive, small (~ 1 cm), raised, spherical, red masses, colloquially referred to as “cannonball” lesions.

3.5.3 Lymphoma

Lymphoma in the spleen is similar to that in other locations (see main section on Lymphoma). There are uncommon forms of primary splenic lymphoma that are generally slow growing and tend to be limited to the spleen (e.g. mantle zone and marginal zone lymphomas), but these will not be covered here. Various leukemias can also enlarge the spleen.

3.5.4 Hemophagocytic histiocytic sarcoma

This is a malignant neoplasm that often manifests with splenomegaly. It is characterized by a diffusely meaty spleen, enlarged by huge numbers of neoplastic macrophages. These macrophages phagocytose erythrocytes, leading to anemia, often mimicing immune-mediated hemolytic anemia. It is primarily a neoplasm of the dog, and may also affect the bone marrow.

3.5.5 Non-angiomatic, non-lymphomatous sarcomas

This is a catch-all term for the large variety of other tumours that can arise in the spleen. They are grouped together because they share a similar gross and histological appearance, and, more importantly, a similar clinical behaviour. They can be broadly divided into two categories: clinically benign tumours, and their nefarious counterparts, malignant and metastasizing masses. **Mitotic rate is the most useful histologic feature in differentiating between the**

two. The survival time for animals with tumours of a high mitotic rate is *greatly decreased* as compared to those with low mitotic rates. The liver is the most common site of metastasis.

3.6 Miscellaneous conditions

3.6.1 Siderotic plaques

Syn: Gamma-Gandy bodies, siderofibrotic plaques.

These are **very common lesions** in the spleen of geriatric dogs, and are thought to be the result of previous trauma or hemorrhage. They are found in the splenic capsule, typically along the periphery but often extending centrally, and are tan to brown to yellow, firm, and dry. Their primary importance is in recognizing that **they are incidental findings, of no clinical relevance.**

3.6.2 Splenic rupture

Rupture of the spleen can be an acute, life threatening event, primarily due to massive hemorrhage and hypovolemic shock. Prompt surgical intervention is generally required. By far, the most common cause of splenic rupture is trauma. Occasionally, splenic rupture is diagnosed in the absence of trauma; in those cases, an underlying cause for abnormal splenic fragility should be sought. In particular, neoplasms or other growths may render the spleen more susceptible to rupture.

An interesting and occasional consequence of splenic rupture is the so-called “seeding” of the abdomen with splenic tissue, which can implant and develop functional capabilities. The presence of multiple small splenic nodules throughout the abdomen is known as **splenosis**. Be cautious, however, when you are faced with interpreting this lesion: metastatic hemangiosarcoma can also present as small red nodules throughout the mesentery, and they are very difficult to distinguish grossly.

3.7 Biopsying the spleen

Finally, a quick note on biopsies of the spleen. When a lesion is discovered on the spleen, the usual clinical course is a complete splenectomy. **The entire spleen is always the best sample to send**, however, it is not always technically feasible. In cases where the whole spleen cannot be sent, taking multiple samples of the **junction of the mass (or lesion) and normal spleen** is the most likely way to obtain a diagnosis. Many of these tumours, especially hemangiosarcomas, have necrotic centers, and sampling the mass alone may result in a dreaded “non-diagnostic” report. Note the word “multiple” above: the

classical recommendation is that *seven* biopsies of a splenic mass are required to reliably rule out the possibility of hemangiosarcoma (though a current paper suggests that perhaps 5 are adequate).

Splenic masses, in particular, often come in multiples. **They are not guaranteed to be the same thing.** Therefore, *each* mass should be biopsied and submitted.

If a diffusely meaty spleen has been removed, send several 1 cm thick samples.

4 Lymph nodes

Lymph nodes are the waystations that lymph passes through on its way back to the thoracic duct and general circulation. They are roughly ovoid or reniform (“bean” shaped), and are present in predictable anatomic locations, some of which are superficial and **clinically palpable**. They are critical tissues in the immune response of the animal to pathogens, representing a major site for antigen presentation and B-cell maturation.

As with all the other organs and systems in this section, a review of the normal anatomy and function of the lymph node is useful. The reference textbook (available electronically from the library) is helpful.

Unlike other organs, where we typically go through diseases by process (i.e. inflammatory disease, neoplastic disease, etc), with lymph nodes it is useful to separate diseases into those that make the lymph node big (lymphadenopathies), and those that don’t. As a rule of thumb, **enlarged lymph nodes are much more significant than small lymph nodes** to the point where “lymphadenopathy” generally refers to enlarged lymph nodes (even though it strictly means “disease of the lymph node”).

4.1 Lymphadenopathy

There are **3 basic mechanisms that lead to enlargement of the lymph node**. You should know them well.

1. Infection of the lymph node itself (lymphadenitis)
2. Reaction of the lymph node to infection (reactive hyperplasia)
3. Enlargement due to neoplastic growth

The number and distribution of enlarged lymph nodes can be a helpful, but not bulletproof, clue to the underlying etiology. For example, enlargement of multiple lymph nodes from various anatomic locations (e.g. popliteal, superficial cervical, and submandibular) is suggestive of neoplasia, whereas enlargement of a single lymph node may be more indicative of lymphadenitis or reactive hyperplasia.

4.1.1 Lymphadenitis

Lymphadenitis refers to a lymph node that is *infected*, which is distinct from a lymph node that is reacting to infection elsewhere - i.e., reactive hyperplasia. You should be able to confidently describe the differences between these two conditions.

Lymphadenitis can be acute or chronic. It can be caseous, suppurative, or granulomatous.

4.1.1.1 Acute lymphadenitis

This may be the result of a regional lymph node draining an active infection, and becoming infected in turn. If the infection is localized, then a single lymph node may be affected. More generalized infections, or septic animals, may have infection of multiple nodes. Affected lymph nodes are enlarged, soft to firm, red, and often bulge on cut section.

Strangles in horses is an important example of acute suppurative lymphadenitis. It is caused by *Streptococcus equi* spp. *equi*. Following inhalation or direct contact with the organism, there is colonization of the lymph nodes, particularly the **mandibular and retropharyngeal** lymph nodes. Large abscesses develop, and may rupture, draining pus and bacteria into the environment or the guttural pouches. In a subset of cases, bacteria disseminate through the lymph or blood to other organs (lung, liver, kidney, mesenteric lymph nodes) and form similar lesions in a condition known as **bastard strangles**.

4.1.1.2 Chronic lymphadenitis

These conditions are characterized by their longer duration and often the presence of fibrosis. A few key diseases are discussed.

Caseous lymphadenitis (CLA) is primarily a disease of small ruminants, though the causative agent, *Corynebacterium pseudotuberculosis*, also causes ulcerative lymphangitis in cattle and horses. CLA in sheep almost always occurs following an injury from shearing. Organisms then spread to local, draining lymph nodes, where it develops into a chronic, suppurative to caseous infection. The disease can then enter a cycle of abscess formation (characterized by a fibrous capsule) followed by necrosis and reformation of an abscess. This creates **characteristic concentric laminations**, considered to be hallmarks of this disease. Once in the lymph node, this infection is often persistent, and can spread slowly to internal organs. In goats, CLA predominantly affects the lymph nodes of the head.

Mycobacterium bovis, the causative agent of bovine tuberculosis, causes single to multiple discrete white areas of *granulomatous* inflammation in lymph nodes. The center of these nodules often exhibits caseous necrosis. Lymph nodes are enlarged and occasionally gritty on cut-section.

Porcine circovirus 2 causes a variety of clinical syndromes, including post-weaning multisystemic wasting syndrome. One manifestation of viral disease is depletion of the lymphocytic population of the lymph nodes, and enlargement with diffuse, granulomatous inflammation.

4.1.2 Reactive hyperplasia

Reactive hyperplasia is the stereotypical, physiological reaction of a lymph node to antigenic stimulation. It usually involves proliferation of lymphocytes and expansion of the cortex or paracortex, but can occasionally manifest as expansion of the sinuses with histiocytes. The number of lymph nodes affected is variable and depends on the location(s) of the initiating stimulus. Grossly, lymph nodes are enlarged, pale, non-painful, and may bulge on cut-section.

Despite the brevity of this section, **this is a very common finding in all domestic species**. Understanding that enlarged lymph nodes are a **normal, immunological reaction to infections** is important.

4.1.3 Neoplasia

4.1.3.1 Primary neoplasia

Lymphoma, lymphoma, lymphoma. **Lymphoma is one of the most common malignant neoplasms of many domestic species**. Whether you practice bovine, equine, or companion animal medicine, you will encounter lymphoma. Chickens? Lymphoma. Wildlife? Usually infectious diseases, but sometimes, lymphoma. It is worth taking your time to work through this section (because you will need to know it, and because it will be on the exam). As a sidenote, lymphoma is sometimes referred to as lymphosarcoma. The two terms mean the same thing, and lymphoma is currently the preferred term.

The classification of lymphomas is a complex, multifactorial process, involving clinical, histopathological, immunohistochemical, and molecular information. In reality, the diagnostic process often ends with the diagnosis of ‘lymphoma’, rather than pursuing the advanced diagnostic modalities that would be commonplace in human medicine. The current classification system was developed by the World Health Organization, and includes around 35 different subtypes. Some practical aspects of classification are useful to review.

1. Anatomical location

- i) Multicentric: present in multiple lymph nodes, ± liver, spleen, bone marrow
- ii) Cutaneous: in the skin
- iii) Alimentary: within the gastrointestinal tract
- iv) Hepatosplenic: in the liver and/or spleen

- v) Thymic: Affects the (you guessed it) thymus.
 - vi) Miscellaneous: ocular, hepatic, specific splenic forms, etc.
2. Immunophenotype: B or T cell, or neither (e.g NK cell).
 3. Grade: based on histopathological features, usually mitotic count. A higher grade is associated with a *poorer* prognosis.

Lymph nodes with lymphoma appear grossly enlarged and homogeneously pale white, occasionally streaked with hemorrhage. They are typically firm. It is difficult to grossly distinguish between reactive hyperplasia and lymphoma.

There are key species differences when it comes to lymphoma.

4.1.3.1.1 Canine

Lymphoma is the most common hematopoietic neoplasm of dogs. Large-breed dogs are predisposed, including Golden and Labrador retrievers and Boxers. Cocker spaniels, Terriers, and Beagles are also overrepresented. Several forms of lymphoma occur in the dog, but **multicentric** is by far the most common, usually presenting as a **generalized lymphadenopathy, especially of the peripheral lymph nodes**. They can be either **B-** (more common) or **T-cell in origin**. Clinical signs are vague and are often absent during the early stages of disease. Alimentary lymphoma occurs but is less common than in cats. They are usually of **T-cell** origin, and often progress slowly. Clinical signs relate to the GI tract: diarrhea, vomiting, and weight loss. Cutaneous lymphomas, most commonly epitheliotropic T-cell lymphoma, are about as common as GI lymphoma. They are often a cause of pruritus, but gross lesions are varied and difficult to distinguish from other causes of pruritus. Epitheliotropic lymphoma may affect the skin or gums (Fig 6). The disease is slowly progressive. A variety of other slowly progressive lymphomas affect the dog, but are infrequent. Chemotherapy is available for many canine lymphomas with varying degrees of success, but can often induce remission.

4.1.3.1.2 Feline

Lymphoma is the most common malignant neoplasm of cats, with alimentary lymphoma being the most common anatomical type. Other forms, such as multicentric, nasopharyngeal, or mediastinal, occur, but with far less frequency. Alimentary lymphoma in cats is usually of **T-cell** origin (formally known as enteropathy-associated T-cell lymphoma). This type of lymphoma typically progresses slowly, with weight loss, diarrhea, and vomiting as the principle clinical signs. The microscopic appearance of the disease is one of infiltrative lymphocytes within the epithelium and lamina propria, and it can therefore be quite difficult to distinguish from inflammatory bowel disease. Submitting full-thickness or deep-endoscopic biopsies

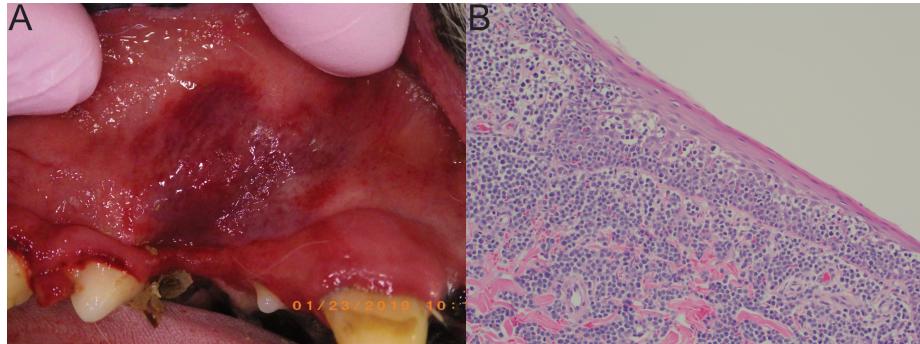


Figure 6: A) A poorly demarcated area of the gingival mucosa is deep red to purple, looking very much like an ecchymosis (bruise). B) Histology of this area reveals a neoplastic population of round cells infiltrating the epidermis and superficial dermis. The diagnosis was epitheliotropic lymphoma.

gives you the best chance at a diagnosis. Many endoscopic biopsies are ‘wimpy’ - consisting of shallow portions of the superficial mucosa, giving the pathologist little ability to distinguish between the two diseases. The prognosis for this type of lymphoma is a bit controversial, as large scale studies are still unavailable, but it is generally considered to be a slowly ‘smoldering’ disease that an animal may live with for a significant amount of time.

Multicentric lymphoma is the next most common form in the cat, and unlike in the dog, **involvement of the peripheral lymph nodes is not a prominent feature**. Instead, liver and kidney are more commonly involved.

Historically, feline lymphoma was **highly associated with the prevalence of feline leukemia virus**, and testing and control of FeLV has greatly reduced the incidence of lymphoma, particularly thymic and multicentric lymphomas, in younger cats. FIV has also been associated with lymphoma.

4.1.3.1.3 Bovine

Lymphoma in cows is separated into two major forms:

1. **Enzootic bovine leukosis:** Caused by bovine leukemia virus (BLV), a retrovirus that infects **B-cells**. The virus is transmitted horizontally, mostly by infected arthropods, iatrogenically (re-use of needles, rectal sleeves), colostrum/milk administration, or natural breeding. The virus causes **multicentric lymphoma in cattle 5-8 years old**. Like all retroviruses, once the virus has infected the host, infection is lifelong, but *infection does not necessarily result in lymphoma*. Of the infected animals, around 30 % will develop a persistent lymphocytosis, and of those, around 5 % will develop lymphoma. Animals that present with

the disease typically have markedly enlarged lymph nodes, nodules in the heart (Fig 7), abomasum, uterus, and the vertebral canal. Clinical signs depend on the extend to which the neoplasm affects the various organs.

2. **Sporadic lymphoma:** these are associated with younger cattle **and are not associated with BLV**. They are usually of **T-cell origin**.

- i) Juvenile, multicentric: Typically found in calves 3 - 6 months old. Virtually all lymph nodes are affected, and as well as bone marrow, leading to myelophthisis and pancytopenia. Visceral organs may be involved.
- ii) Thymic: Typically in cattle < 2 years old, thymic lymphoma may cause pre-sternal swelling leading, jugular distension, and local edema. As the tumour grows, compression of the lungs may lead to respiratory difficulty.
- iii) Cutaneous: Typically in cattle 2 - 3 years old, this is the **least common form of lymphoma**. Presents with round, raised, plaques along the head, sides, and perineum that often ulcerate. The disease may wax and wane, but eventually progresses to involve visceral organs (indistinguishable from enzootic bovine leukosis).

4.1.3.1.4 Equine

Lymphoma is the most common malignant neoplasm of the horse. Most are **B-cell** in origin and are multicentric. Note that in the horse, the multicentric form typically forms masses in the abdomen and thorax, as well as the subcutis, but **not typically a generalized lymph adenopathy**. Alimentary and cutaneous forms also occur, and both are usually T-cell in origin. Horses with any form of lymphoma will typically present with clinical signs of ill-thrift, anorexia, depression, and pyrexia. Colic may occur with alimentary or multicentric lymphoma. Quarter horses and Thoroughbreds most often develop multicentric lymphoma, while Standardbreds have a higher incidence of alimentary lymphoma.

4.1.3.1.5 Porcine

Lymphoma is the most common neoplasm in pigs, but data is lacking regarding the most common type. Multicentric (affecting visceral, rather than peripheral, lymph nodes) and thymic are most common. Affected animals are often young (< 1 year).

4.1.3.1.6 Avian

Poultry are affected by two different forms of lymphoma. Both are caused by virally-induced malignant transformation of lymphocytes.



Figure 7: In this example of bovine leukosis, there are multiple raised white nodules throughout the myocardium of this heart (you are looking into the opened left atrium and ventricle). Photo courtesy of J. Schenkels.

1. **Marek's disease** primarily occurs in younger birds (four weeks and older), and is of **T-cell origin**. It is caused by Gallid herpesvirus 2, for which a vaccine is available. It causes lymphoma in a wide variety of organs, but particularly prominently in the nerves (e.g. sciatic nerves), leading to the classic clinical sign of uni- or bilateral hind-limb paralysis. The eyes, visceral organs, and skin are often also affected, but the *bursa of Fabricius is usually spared*.
2. **Avian leukosis** is caused by a retrovirus and transforms **B-cells**. Due to the nature of the virus, disease does not usually manifest until the animal is older than 14 weeks. The virus causes lymphoma in a variety of organs, including the bursa of Fabricius, liver, spleen, and kidney. Distinguishing between avian leukosis and Marek's disease in an older chicken is challenging with histopathology alone and generally requires ancillary diagnostics.

4.1.3.2 Metastatic

The lymph nodes are a very common site for metastases from a wide variety of neoplasms (carcinomas, mast cell tumours, oral malignant melanomas, for example). The gross appearance of the lymph node will vary somewhat depending on the primary neoplasm, but in general they are enlarged, firm, and may be mottled on cut section. Metastases will appear first in the closest draining lymph nodes.

4.2 Lymph node atrophy

As noted above, small lymph nodes are uncommonly a sign of pathology. Primary causes of small lymph nodes include:

1. Atrophy associated with aging (senile atrophy)
2. Cachectic atrophy, seen in animals

5 Thymus

The thymus is responsible for the training of T-cells, ensuring that self-reactive T-cells are removed from circulation. The thymus is at its largest and most active in neonatal and young animals, where it forms a large, multilobular, intrathoracic organ in the cranial mediastinum. As you'll recall, the thymus regresses over the lifetime of an animal, becoming practically inapparent by the time most animals have reached sexual maturity. Although still present, at this stage the thymus is typically indistinguishable from the mediastinal fat.

It is broken down into lobules composed of a cortex and medulla, and has both an epithelial and lymphoid component.

With the exception of a few diseases, thymuses tend not to be a primary source of clinical disease.

5.1 Miscellaneous conditions

5.1.1 Atrophy

Thymic atrophy is one of the most common changes noted in the thymus. Two basic pathogenic mechanisms can lead to thymic atrophy. Because the lymphoid population of the thymus is composed of precursor cells from the bone marrow, destruction of precursor cells in the bone marrow can lead to atrophy of the thymus. The second mechanism is direct damage to the lymphocytes within the thymus itself. Causes include viral infection (e.g. BVDV, canine distemper virus, FIV, canine and feline parvovirus, EHV-1, and classical swine fever virus), toxins, chemotherapy, and radiation. Care must be taken when deciding if the thymus is atrophied: the thymus naturally involutes with age, and thus age-matched controls are often necessary to grossly identify atrophy.

5.1.2 Hypoplasia

Recall that aplasia signifies failure of an organ to reach its normal size (contrast this with atrophy, in which organ has at one point been normal, but has then become smaller). Aplasia of the thymus is a congenital defect, usually involving an immunodeficiency of T cells. The most common manifestation of thymic hypoplasia is part of *severe combined immunodeficiency* syndrome in foals, particularly Arabians, and certain breeds of dogs (Jack Russell terriers, Bassett hounds). The thymus of these animals is devoid of lymphocytes, rendering them markedly small. These animals are predisposed to infection and frequently do not live long after birth.

5.1.3 Hemorrhage

Massive thymic hemorrhage is an uncommon but important finding particularly in dogs, where it can be the only lesion. Three main causes are implicated, and should be included in a differential list:

- Anticoagulant rodenticides
- Trauma
- Idiopathic

These three causes are indistinguishable on gross examination, and would require additional history and/or toxin testing. The degree of hemorrhage can be profound and life threatening.

5.2 Thymic neoplasia

The thymus has an **epithelial and a lymphoid component**, and either may give rise to a neoplasm. Neoplastic proliferations of lymphocytes are of course lymphomas, while in the thymus, epithelial neoplasms are known as thymomas.

5.2.1 Thymic lymphoma

Unsurprisingly, thymic lymphoma is almost always T-cell in origin. It usually affects younger animals, particularly **cats**, **calves**, and less commonly dogs. **In young cats, thymic lymphoma is highly associated with feline leukemia virus (FeLV)**, and the widespread vaccination of cats against this virus has dramatically decreased the incidence of the disease in these animals. There is no viral association in other animals, or in older cats.

Grossly, the neoplasm is generally present diffusely throughout the thymus, creating a large, space-occupying mass in the mediastinum. The mass may get large enough that it compresses the lungs, resulting in dyspnea. Thoracic effusion is also frequently present.

5.2.2 Thymoma

This neoplasm of the epithelial component of the thymus is most frequently seen in dogs and small ruminants (sheep, goats). They tend to be slow-growing, relatively benign tumors that only rarely metastasize. They are grossly indistinguishable from thymic lymphoma, thus, **histopathology is required to differentiate between the two**. Dogs with thymomas often develop myasthenia gravis (note: link takes you to a separate package of course notes on skeletal muscle. The details of myasthenia gravis are *not* required for exams on the hemolymphatic system).

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