NationWide LABORATORIES

Getting the best from your pathologist. Post-mortems and surgical biopsies. Tips and tricks for vets in practice

Part I

POST-MORTEMS

PRESENTED BY

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Part I

POST-MORTEMS

Introduction

Post-mortem examination (PME) examination is the gold standard by which to confirm the cause of death and are essential in providing quality control to medical practice. Studies have reported discrepancy rates between clinical diagnoses and PME of between 14.9% and 39.7%, highlighting the importance of PME in confirming the accuracy of clinical diagnosis (2, 5). Another study reported disagreement of 26% between the ante-mortem clinical diagnosis and the PME findings (13). The Royal College of Veterinary Surgeons lists PME as a Day One Competency, indicating that all veterinary surgeons should be able to carry out this procedure as well as record observations, take samples, store and transport them (11).

When/when not to carry out PME

Situations in which PME should or could be carried out in practice include cases of sudden unexplained death (SUD), to confirm a clinical diagnosis, or to investigate complex cases in which a final diagnosis was not made ante-mortem. Carrying out PMEs on these occasions are an opportunity for learning for the veterinarian, as well as an opportunity to provide a useful service to clients by informing them as to the cause of death of their pet(3,8). Post-mortem examination may give clients reassurance that they “did the right thing” in cases where euthanasia was elected. In cases of sudden or unexplained death, PME may provide closure to pet owners, for example in cases where anundiagnosed underlying condition that lead to death was present (e.g. a puppy with a congenital heart defect).

Situations in which PME should be avoided by practitioners include forensic/legal cases (for example, suspect deliberate intoxication or death of an animal in boarding kennels) as should the case be taken to court, testimony as to the cause of death should ideally be provided by a qualified pathologist as an expert witness. PME on cruelty/welfare cases should be avoided for the same reason. Practitioners should avoid PME on animals if death occurred while the animal was under their care (e.g. a healthy animal that died during a routine surgical procedure such as neutering/dental or post-vaccination), given the conflict of interest that would arise should the owner decide to pursue litigation (3,8). In this situation, PME should be carried out by a different veterinarian and this option should be offered to clients (12,8). Necropsy of very large animals (e.g. large or giant-breed dogs) should be avoided given the requirement for space and equipment which may not be readily available in a first opinion veterinary clinic.3 If there is a suspicion of infectious/zoonotic disease (e.g. parvovirus, Salmonellosis, tuberculosis) suitable containment facilities are required which may not be available in a first opinion practice.3 Finally, cases of neurologic disease often involve removal of the brain and/or spinal cord which are specialised procedures that require training and expertise as well as specialised equipment (e.g. oscillating saws). These cases should ideally be referred for post-mortem by qualified anatomic pathologists.

Before PME

Prior to carrying out a PME, clear communication with the client is essential. Some owners may be reluctant to opt for PME due to religious or sentimental reasons and the owners wishes should be respected first and foremost. Permission should be obtained, ideally with a signed consent form.3 Verbal consent is however acceptable.12 The owner should also be informed if samples are taken for further testing (e.g. histopathology) and consent for this should also be obtained. The owner should be informed of what the procedure involves and the cost. A discussion of how the remains will be dealt with is essential, with cremation likely being the most suitable option.3 Detailed guidance of communication and consent are available on the RCVS website (10). In cases of sudden unexplained death in which the vet has not recently been involved in treatment of the animal, a clinical history should be obtained from the owner. This should include details such as signalment, previous health conditions, ante-mortem clinical signs, medications, the location and time of death, whether or not the death was witnessed, and if death was accompanied by unusual clinical signs (e.g. seizures, dyspnoea, vomiting etc) (3,8). Post-mortem examination should be carried out as soon as possible (ideally within 24 hours of death). Prior to PME the owner should be advised regarding storage of the body. Freezing should be avoided as freezing artefacts may significantly affect both macroscopic and histologic findings. Ideally the body should be stored at refrigeration temperature (i.e. between 1 and 4 degrees Celsius). The owner should also be instructed to be mindful of biosecurity concerns (e.g. to wear gloves when handling the body, to avoid contact with body fluids) (3).

Equipment for PME

The following equipment is necessary for performing a PME in most cases: PPE (gloves, apron, shoe covers, scrubs); weighing scales; scalpel; forceps; scissors; pm knife; cutting board; pruning shears; bone forceps; note taking equipment; camera; measuring jug/bowl; syringes; sterile swabs; pots with formalin.

Scalpels, forceps and scissors can be ordered from surgical suppliers. Secateurs can be purchased from hardware shops (e.g. B&Q), and knives can be obtained from websites supplying butcher's equipment (e.g. www.butchersequiment.co.uk). Bone forceps may be obtained from laboratory equipment suppliers (e.g. www.thermofisher.com). Post-mortem examination of very small animals (e.g. cats, puppies) may not require larger items such as secateurs, bone forceps or post-mortem knives but these will usually be required for medium-large animals and older animals.

Preliminary examination

Prior to beginning the dissection process, a preliminary examination should be carried out (3,8). The body should be weighed, as this is important for interpreting organ weights and can be compared to previous weights recorded at the veterinary clinic to ascertain if significant weight loss/gain has occurred. Radiographs may be useful in cases where a road traffic accident is suspected, as assessing the skeleton via dissection can be difficult. This is especially true for bones that are surrounded by large amounts of muscle tissue (e.g. vertebrae). A microchip scan should be carried out to confirm the identity of the animal. Similar to a physical examination on a living animal, the hair, skin, eyes and orifices should be examined for abnormalities, lesions and discharges (3,8). The skeleton should be palpated in case of fractures/luxations although rigor mortis may render this difficult. Mucous membrane colour should be assessed, bearing in mind that pooling of blood on the dependent side of the body may mimic anaemia/congestion.

Time of death

Assessment of the time of death is very difficult. Rigor mortis may be used to formulate an approximation. The onset of rigor is between two and six hours post-mortem, full rigor is achieved between six and 36 hours, and rigor resolves after 36 hours (1). However, given the many factors influencing rigor mortis (e.g. ambient temperature, body temperature, body

condition, etc) this is not an accurate method to determine time of death. No accurate method of determination of time of death has been standardised in veterinary medicine (1).

Record keeping and photography

Notes should be taken during the PME describing the main findings. An assistant or voice recording device may be required to do this hygienically. Lesions should be described in terms of colour, shape, texture (smooth, rough, friable, etc), contour (raised, depressed, nodular etc) and distribution (focal, multifocal, diffuse, focally extensive etc). The size of lesions or percent of organ affected by a lesion should be noted. A ruler/measuring take should be used to take measurements where appropriate. Lesions should be described but not interpreted, as very often, histologic examination is needed for full confirmation of the pathologic process (3,8). Photographs may be taken to more accurately record key findings.3,8 These can be emailed to the pathology lab should histopathologic examination of tissues be carried out, as it is useful for the pathologist to view the gross PME findings. If taking a photo of a lesion in-situ within the body, a photo should be taken from a distance with anatomic landmarks, and then “close up” to obtain more detail of the lesion. If organs are photographed after removal, they should be cleaned of blood or other fluids using tissue paper and placed on a clean background (e.g. cutting board) with a ruler to lend scale to the picture, especially if a size increase/decrease is notable. If one of a set of paired organs is abnormal, the pair should be photographed together for comparative purposes.

PME tips

These notes are not intended to be a step-by-step guide to carrying out a PME and the reference list provides useful resources on this topic (3,6,8). However, the following advice may be useful: positioning the body in either lateral or dorsal recumbency is acceptable, but lateral recumbency offers superior stability of the body while dissecting. All solid organs (e.g. lungs, liver, kidneys, endocrine glands, spleen) should be removed incised at regular intervals to inspect the parenchyma for abnormalities within the tissue that are not visible from the surface (8). All tubular/hollow organs should be removed and opened (e.g. GIT, trachea, urinary bladder, uterus) to inspect the contents (8). The heart is a complex organ and various dissection techniques may be employed (3,9). However, all four chambers and the great vessels should be opened, and the valves inspected for increased thickness or irregularities. The liver and heart should be weighed, and their percentage of the total body weigh calculated. The canine heart should comprise less than or equal to 0.7% body weight while the feline heart should weigh less than 17g (9). Measurements should be taken of the thickness of the left and right ventricular free walls and the interventricular septum. The ratio of thickness between the right ventricular free wall, the interventricular septum and the left ventricular free wall should be 1:3:3.3 The liver should represent 3-4% of the total body weight (8).

Post-mortem changes

It should be borne in mind that post-mortem changes may mask or mimic lesions (4). Recognition of these changes requires experience at carrying out PME and this may be difficult for clinicians who are not regularly carrying out this procedure. Common changes include clotting of blood in the vessels and heart, pooling of blood due to gravity on the dependent side of the animal (livor mortis), and autolysis which is especially pronounced in non-sterile tissues or those exposed to pancreatic enzymes or bile. Bacterial overgrowth will accelerate autolytic changes. Animals euthanased by barbiturate injection may develop grey-tan coloured, rough, speckled lesions on the inner wall of the right ventricle (4). Gas distension of the GIT may mimic gastric dilatation.

Sampling for histopathology

Samples of tissue may be taken for histologic assessment in a pathology lab. Ideally, samples should be taken from the lungs, liver, kidneys, heart, spleen, pancreas, GIT

(duodenum, jejunum, ileum, colon) and from all lesions seen grossly (e.g. masses)(3). If no obvious lesions are observed, brain should also be submitted, although as previously mentioned, removal of the brain may not be possible given the specialised nature of this procedure, especially in large/giant breed dogs. Tissue samples should be approximately 10mm (3) and should be placed in a container of neutral buffered formalin with a ratio of tissue to formalin volume of 1:10. If the clinician is uncertain as to what tissue should be submitted, extra samples can be taken and stored in formalin and a discussion of the case with the pathologist may help clarify what tissues should be submitted. Samples taken from the different regions of the gut should be placed in separate containers of formalin and labelled as histologic distinction between the various areas of the GIT can be difficult histologically. If samples are taken 24-72 hours after death, artefacts such as gas bubbles, saprophytic bacteria, and loss of tissue architecture will occur and there is also a loss of tissue stain uptake, rendering histologic interpretation of findings difficult (4). Therefore, the PME should be carried out as soon as possible after death.

Sampling for other tests

If intoxication is suspected, samples of blood, urine, tissue (liver, kidney,lung, and fat), and stomach content should be obtained for analysis (3). These can be stored in ziplock bags (solid tissues) and pots (liquids) and frozen. These can be submitted for toxicological analysis after histology has been carried out if this is suggested by the pathologist. Faecal samples may be obtained for analysis in cases where parasitaemia is suspected (3). Smears for cytology can be prepared for in-house examination or for submission to the lab (3). If slides for cytology are being submitted to the laboratory, these should be posted separately to the samples in formalin, as formalin fumes cause artefactual changes to cells present in cytology samples. Tissue samples, swabs or fluid aspirates may be submitted in sterile pots for microbial culture. Sample should be taken in a sterile fashion with care to avoid contamination (e.g. by the GIT content). Samples may also be taken for PCR analysis in cases where certain infectious diseases are suspected (e.g. canine herpesvirus). The lab should be consulted as the appropriate tissue to samples for PCR testing.

Causes of sudden unexpected death

The most common causes of SUD in any species include adverse drug reactions, anaphylaxis, anaesthetic death, bacterial sepsis, drowning, electrocution, exsanguination, heat stroke, intestinal strangulation, physical trauma and intoxication (4). The commonest causes of SUD specific to dogs include cardiac anomalies, gastric dilatation and volvulus, haemorrhage from atrial or splenic haemangiosarcoma, hypoadrenocorticism (Addison's disease), parvoviral infection and pulmonary arterial thrombosis, and in cats, hypertrophic cardiomyopathy and feline parvovirus infection (4). Causes of SUD in small animals have been reviewed and practitioners should be aware of the possible differentials for these cases (8). In some cases, no obvious lesions will be observed at PME (7). This includes certain toxins (e.g. sodium fluoroacetate, cyanide, mushrooms, strychnine, carbon monoxide), certain cardiac diseases (e.g. syncope, breed associated dysarrhythmia), perinatal death (hypoglycaemia, hypothermia), post-vaccine reactions, anaesthetic death, heat stroke, CNS disease, anaemia and electrocution. Unfortunately, in these cases a final cause of death may not be obtained even if a thorough PME has been carried out by a qualified anatomic pathologist.

Conclusion

Post-mortem examination is listed as a Day One Competency by the RCVS. Therefore, all veterinarians are expected to be able to carry out this procedure and collect appropriate samples. Post-mortem examination can be a useful service to offer clients in the case of SUD and a useful learning opportunity for vets. Vets should familiarise themselves with basic PME technique, sampling and also with causes of SUD in small animals.

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Part II

SURGICAL BIOPSIES

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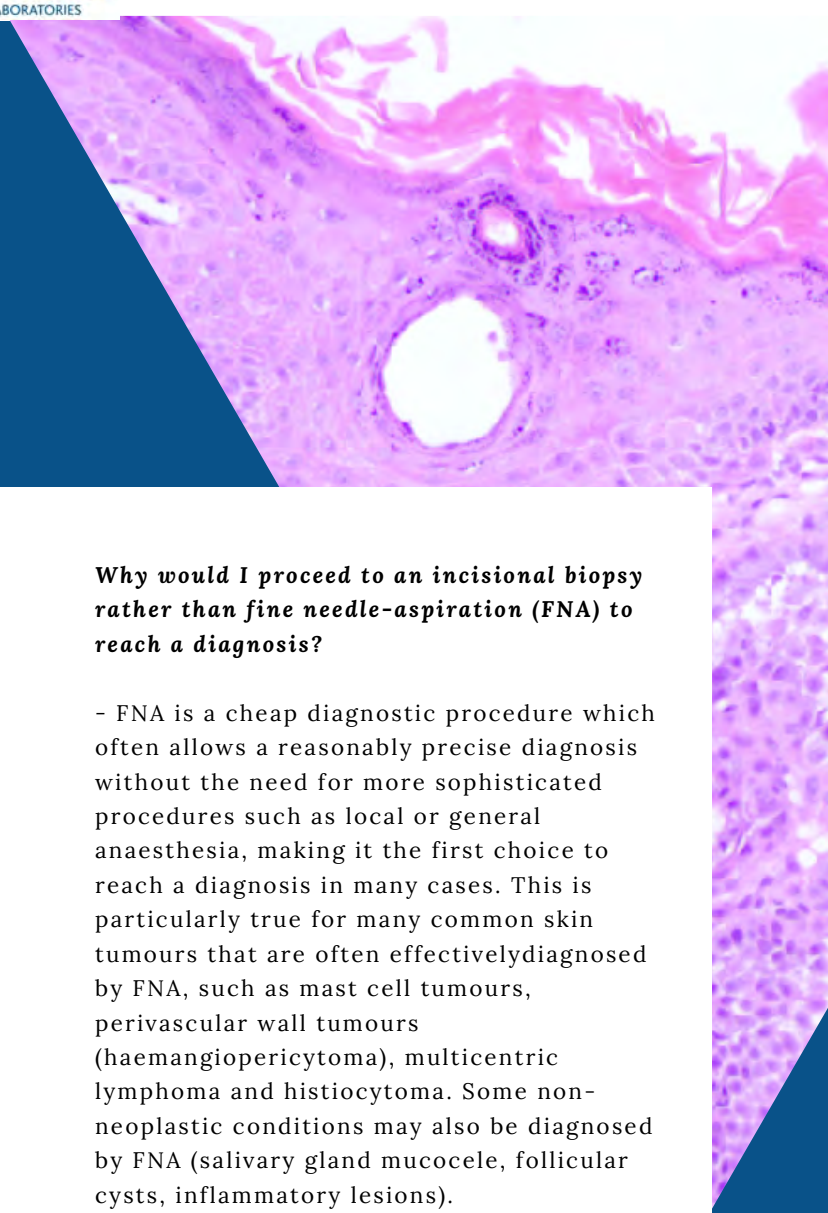

Part II

SURGICAL BIOPSIES

RECOMMENDATIONS FOR SAMPLE COLLECTION FOR SURGICAL PATHOLOGY IN DOGS AND CATS

Types of biopsy in surgical pathology

1. Excisional biopsy

We call a biopsy an excisional biopsy if there is an attempt to remove the whole lesion/mass by the surgeon. This could be either a conservative/marginal excision (no surgical margins), excision with margins (grossly normal tissue is also removed in addition to the mass), or more radical procedures (such as limb/digit amputation, splenectomy, liver lobectomy). This has not only diagnostic but also curative intent.

2. Incisional biopsy

We call a biopsy an incisional biopsy if only a small sample is removed from the lesion/mass, in an attempt to obtain a diagnosis, before deciding what sort of treatment will be used. This could be a punch biopsy (more commonly used for dermatological lesions), wedge biopsy, debulking biopsy or tru-cut/core biopsy (used more commonly to sample viscera or bone lesions).

Regardless of whether a sample from a PME, incisional or excisional biopsy is submitted, it is imperative that all relevant clinical history and differential diagnoses are communicated to the pathologist, as this information is often extremely helpful in forming and eliminating differential diagnoses. We emphasize that it is preferable to send the pathologist a reasonably objective summary of the recent history rather than several pages with a complete record of all history of the patient.

Why would I proceed to an incisional biopsy rather than fine needle-aspiration (FNA) to reach a diagnosis?

- FNA is a cheap diagnostic procedure which often allows a reasonably precise diagnosis without the need for more sophisticated procedures such as local or general anaesthesia, making it the first choice to reach a diagnosis in many cases. This is particularly true for many common skin tumours that are often effectivelydiagnosed by FNA, such as mast cell tumours, perivascular wall tumours (haemangiopericytoma), multicentric lymphoma and histiocytoma. Some non-neoplastic conditions may also be diagnosed by FNA (salivary gland mucocele, follicular cysts, inflammatory lesions).

- Unfortunately, not uncommonly, samples obtained by FNA may not yield enough cells to allow a precise diagnosis to be made. This is not necessarily due to technical limitations of sample collection by the operator, and may be due in fact to the nature of the lesion. Many lesions do not exfoliate enough cells when aspirated to allow an unequivocal diagnosis to be made. Additionally, even in adequate samples with high cellularity, the pathologist may occasionally not be able to differentiate a benign tumour from a malignant one, and the offered diagnosis may have some degree of uncertainty. That is because tissue architecture cannot be appreciated on cytological samples, and this architecture often offers more clues to the pathologist as to the nature of the lesion to be diagnosed, allowing a more precise diagnosis and prognostication.

- If a radical surgical procedure is anticipated, such as maxillectomy/ mandibulectomy for canine oral melanoma, limb amputation for osteosarcoma, or intestinal resection for intestinal GIST, the degree of uncertainty from a cytological diagnosis may be unacceptably high, and the surgeon might opt to obtain an incisional biopsy before decidingon a treatment option. This is because samples obtained for incisional biopsy are expected to have a higher accuracy than those from cytology. As such, if the degree of uncertainty of the cytological diagnosis is deemed high by the surgeon, it would be reasonable to proceed to incisional biopsy before a more radical procedure is performed.

Common practical situations and advantages/limitations of incisional biopsies

Here I will emphasize some common situations occurring in our laboratory to emphasize ways to improve diagnosis and also to highlight limitations of incisional biopsies. I will address specific examples of common diseases.

Feline alimentary lymphoma

This is the most common presentation of lymphoma in cats, and it is most commonly a small cell/low grade lymphoma.We often receive biopsy samples from mesenteric lymph nodes as an attempt to diagnose alimentary lymphoma in cats. While we can occasionally diagnose lymphoma from small samples of lymph nodes, more often than not these are nondiagnostic. This is because overt colonization of mesenteric lymph node by neoplastic lymphocytes happens only in an advanced stage of the disease, and most pathologists would agree that it is difficult to reach an unequivocal diagnosis of small cell lymphoma if there are only few neoplastic cells infiltrating the node (Figure 1). This is supported by some studies reporting lymph nodes rendering a diagnosis of low grade lymphoma in only 33.3 to 59% of cases of feline low-grade alimentary lymphoma, as compared to 83% to 100% obtained by sampling different portions of the intestinal tract (4) (11).

As such, as a rule of thumb, we recommend that samples from the intestinal tract are also sampled in addition to the lymph node. Ideally, this should be at least one sample from each portion of the gastrointestinal tract (duodenum, jejunum, ileum, colon), and this could be done with a punch biopsy. Of special importance is the sampling of jejunum and ileum portions,which should be prioritized, since these are the sites that are more commonly affected (4) (11). Some also like to seize the moment and include also samples from other organs, such as liver, spleen and pancreas, to also allow for the evaluation of neoplastic infiltration in other organs or diagnosis of other concurrent/non-expected illnesses.

Splenic masses in dogs

Splenic haemangiosarcoma (HSA) is the first thing that comes to mind whenever one sees a bloody mass in the spleen. Unfortunately for the pathologist, splenic HSAs not uncommonly are associated with huge haematomas, and this may make a diagnostic assessment difficult, as most of the volume of the mass will be occupied by blood (Figures 2 and 3). It is not uncommon for the pathologist to have to examine multiple sections of the splenic mass in order to offer an unequivocal diagnosis of HSA. Unfortunately, a small degree of uncertainty always remains when a pathologist signs out a report of splenic haematoma, and this is in fact one of the most common reasons for surgeons ringing the pathologist and asking for a case review.

Indeed, this concern has been addressed in current veterinary literature (6) (12). One study reported that up to 11% (4 out of 35) cases of splenic haematoma diagnoses might have underdiagnosed an underlying malignancy, a conclusion supported by evidence of a metatastic malignancy on follow-up (12). In this context, a more recent study indicated that a higher diagnostic accuracy could indeed be obtained if more sections of the spleen are analysed by the pathologist (6).

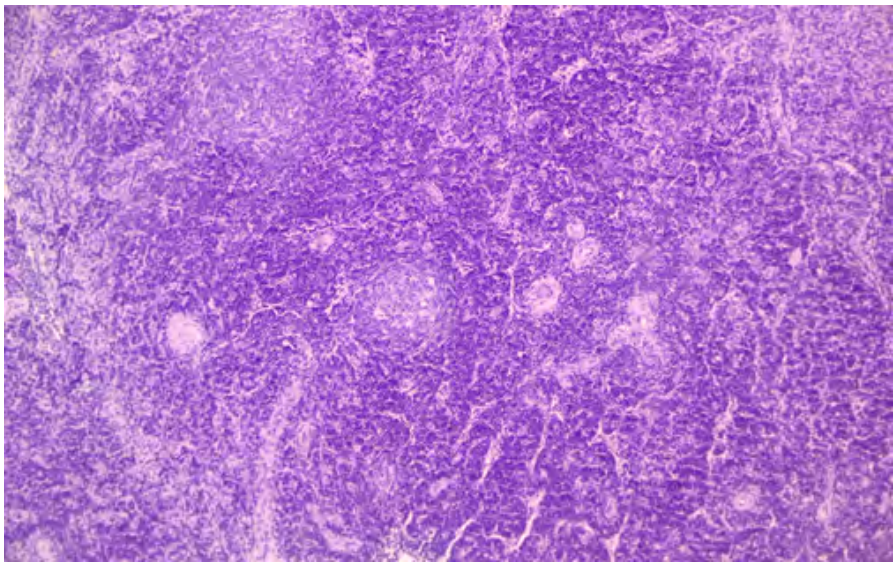

Figure 1. Mesenteric lymph node (cat). This is the mesenteric lymph node of a cat with intestinal small cell lymphoma. This is to emphasize how it is difficult to diagnose lymphoma at this site in cases of alimentary lymphoma. While it is true that there is an atypical expansion of paracortex and medullary cords by small cells, there is also a somewhat preserved nodal architecture, and few follicles are still present. Replacement of normal nodal architecture by diffuse infiltrates of neoplastic lymphocytes is one of the main criteria pathologists use to diagnose lymphoma, and if it is not highly disturbed, most pathologists will not be able to offer an unequivocal diagnosis without risking overdiagnosing lymphoma. That is why it is important to also sample portions of the intestinal tract when alimentary lymphoma is suspected.

As such, although submitting the entire spleen to the lab might not be feasible at times, we recommend that the whole organ is at least kept in formalin until the case is reported. With this approach, additional samples of the lesion can be submitted if the report is equivocalor negative for malignancy. Additionally, as an attempt to increase diagnostic accuracy, from the surgeons point of view, it is always important to also obtain samples of liver, which is the most common site to which splenic HSA spreads. This is especially important if there are grossly visible lesions in the organ. This is because metastatic lesions are usually less disturbed by haematoma formation, making the diagnosis easier for the pathologist. Additionally, in cases where the mass is indeed proven to be just a splenic haematoma, histopathological analysis of liver and omental lesions (grossly akin to metastases) is useful to detect other conditions that may mimic HSA grossly at these sites (such as splenosis or liver telangiectasia) and to give reassurance to the surgeon and pet owner.

Liver masses in dogs

Incisional biopsy of a liver mass is an invaluable procedure, but it is important to understand its limitations. If properly sampled, it is often possible to define whether a hepatic mass is a hepatocellular neoplasia or not. However, these samples are usually sent in the hope of obtaining a definitive diagnosis of malignant versus non-malignant tumour. Unfortunately, for hepatocellular tumours, this is a distinction that is commonly difficult to impossible to be made in small samples. This is because most hepatocellular carcinomas in dogs are well differentiated tumours. This means that neoplastic cells resemble those from adenomas and normal liver, and small samples usually do not allow a highly accurate evaluation of other features of malignancy, such as tissue invasion.

However, from the point of view of the surgeon, a diagnosis of well-differentiated hepatocellular tumour may be all that is needed for further management of the case.

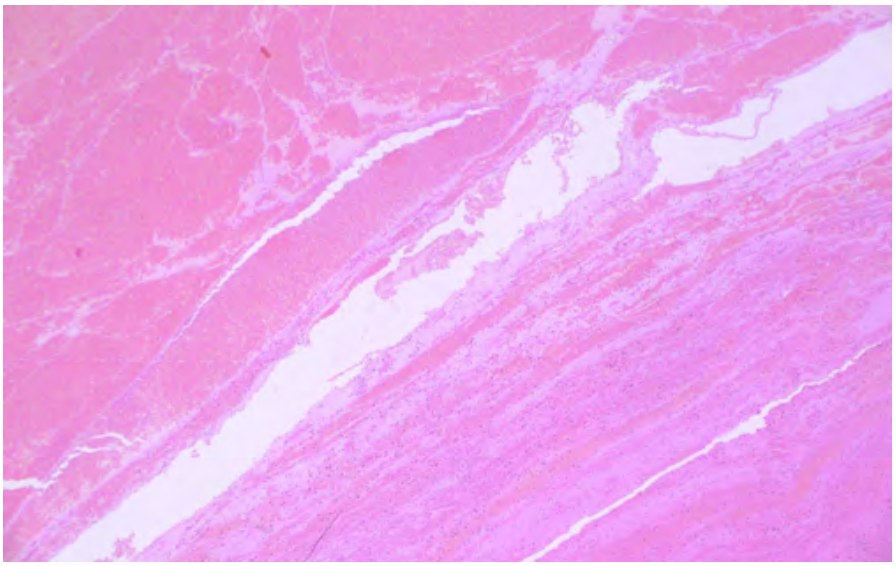

Figure 2. Splenic haemorrhage (dog). This picture shows the exaggerated amount of haemorrhage that is often present in samples from spleen. This particular case is from a HSA, but in this area there are no neoplastic cells to allow this diagnosis to be made. If not enough splenic tissue is evaluated, a diagnosis of splenic haematoma might be rendered even though the patient does have a HSA.

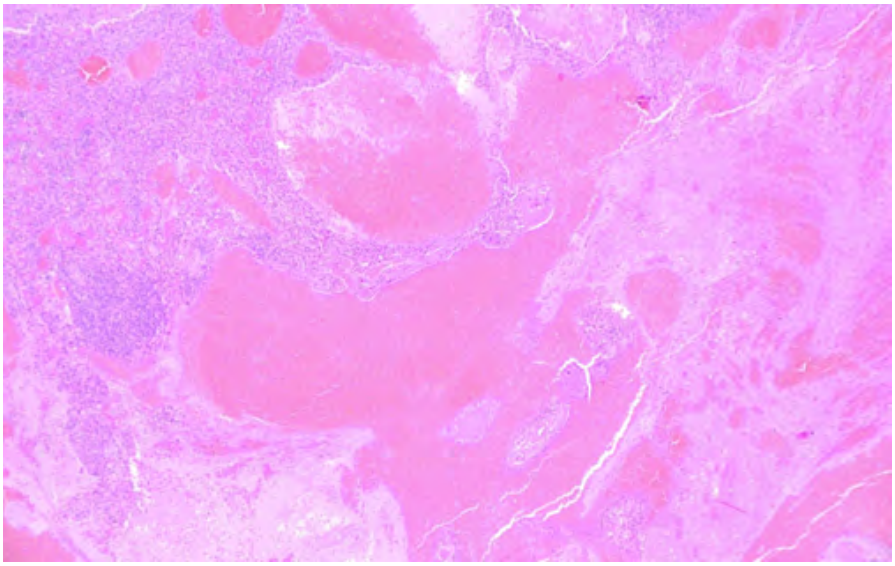

Figure 3. Splenic haemangiosarcoma (dog). This is from the same sample as the previous figure. This is also a highly haemorrhagic tissue. However, in the top-left corner of this picture, it is possible to appreciate neoplastic cells, and a diagnosis can be offered. As you can see, HSA can be present in only very limited areas of a splenic nodule, and that is why pathologists often stress the need of evaluating multiple samples of the mass to be reassured (we often say that it is more comfortable to make a diagnosis of HSA than splenic haematoma).

This excludes other diagnoses that would not be treated by excision (such as hepatic lymphoma, hepatic carcinoid, metastatic neoplasia, cirrhotic liver with macronodular regeneration). After this diagnosis, the decision to treat surgically with lobectomy will depend upon the distribution of the tumour within the liver (massive vs. nodular vs. diffuse), and this is assessed with imaging studies. It has being shown that even for malignant hepatocellular tumours in dogs lobectomy may yield prolonged survival times comparable to benign tumours (> 5 years) if it presents as a massive pattern of distribution (8).

Liver diseases
Liver biopsy has an advantage over cytology because it allows the evaluation of the architecture of the organ, and in a case of inflammatory disease, it is the only way to properly classify it (chronic hepatitis vs. acute hepatitis vs. cholangitis)(Figures 4, 5 and 6). Sampling technique varies, but these are more often obtained via percutaneous (ultrasound-guided) tru-cut or via laparotomy.

Particularly in cats, if a percutaneous tru-cut approach is chosen, it is advised to avoid using an automatic tru-cut biopsy gun device, as these have been suggested to cause vagotonia and an unacceptably higher proportion of fatal shock when compared to a semiautomatic one (16). The risk of bleeding complications should nevertheless be considered, regardless of the device, especially if patients with significant liver disease are selected, and this was reported to occur in up to 15 to 20% of dogs and cats undergoing percutaneous biopsy (3, 13). As such, it is advised to run a coagulation profile prior to the procedure as this has been reported to predict bleeding episodes (3).

If a laparotomy approach is chosen, tissue can be obtained with a punch (ideally a 8mm punch) or a wedge biopsy. It is important that hepatic parenchyma deeper than 5mm and ideally 10mm to the capsule is obtained. This is because liver parenchyma under the capsule often has a higher degree of fibrosis when compared to the average of the whole organ, and this type of sample could thus overestimate the stage of liver disease. This concern is commonplace in medical literature (15), but it is also assumed to be true for veterinary patients (10).

Regardless of the chosen technique, it is imperative that multiple liver lobes are sampled, as it has been shown that, even for diffuse liver diseases, there is a significant variation of lesion distribution throughout the liver lobes (sampling of at least 2 lobes was suggested in one study (7). In this context I also emphasize that we also rarely receive post-treatment biopsies as an attempt to assess success of therapy. While this would appear a sound reasoning, given this expected variation of distribution of a given lesion, it is often difficult to impossible to ascertain, for a single case, whether a given change (as compared to previous biopsies) is reflective of improvement / worsening of the disease, or merely representative of a variation of lesion distribution/intensity throughout different liver lobes/areas. As such, until future evidence indicates that the benefits of protocol biopsies of liver for disease monitoring outweigh the risks, we assume that, unless a new/different condition is suspected, monitoring liver enzymes might suffice, an approach that has been used in current literature (22).

Gastrointestinal tract (endoscopy vs. biopsy)
Whether to approach the gastrointestinal tract via endoscopy or biopsy is commonly a matter of personal preference, but it also depends upon the case being considered. Endoscopy may allow removal of foreign bodies if that is anticipated, and also allow the observation of mucosal lesions, so that significantly affectedareas are sampled. It does however give only limited access to the gastrointestinal tract (stomach, duodenum, large intestine and ileum), and deep mural lesions are not ableto be sampled. Conversely, in a laparotomy approach, although mucosal visualization is not possible, it allows sampling of all layers of the gastrointestinal tract. Particularly in cats, in which the diagnosis of small cell lymphoma is a common concern, this is especially useful, since submucosal/muscular infiltration by small lymphocytes is one important diagnostic criterion for the pathologist (Figures 7 and 8). It also allows the observation of gross lesion in other organs of the abdominal cavity, and this is especially important for conditions that may clinically mimic primary gastrointestinal disease (such as pancreatitis, hepatitis). It does however carry a minor risk of complications, especially in patients with panhypohypoproteinemia, and also a longer recovery time (5).

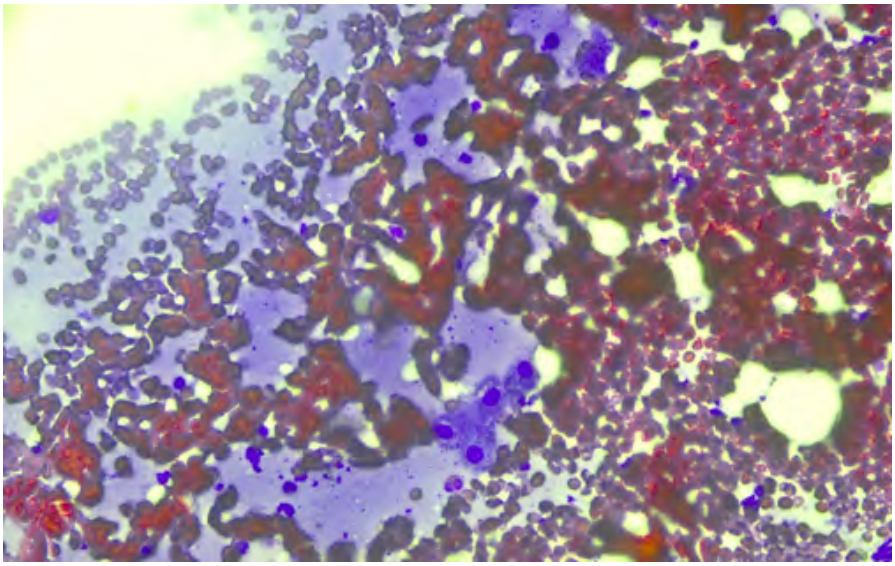

Figure 4. Liver, cytology. In this cytological sample, there are some hepatocytes, and there are few inflammatory cells (mainly neutrophils). However, given the large amount of red blood cells in the background (which is a common finding in cytological samples of liver), it is difficult or impossible to tell whether these cells represent true inflammation of the organ or circulating leukocytes from blood (in which case they would represent only blood contamination of the sample). Thus, cytology of liver gives only limited information

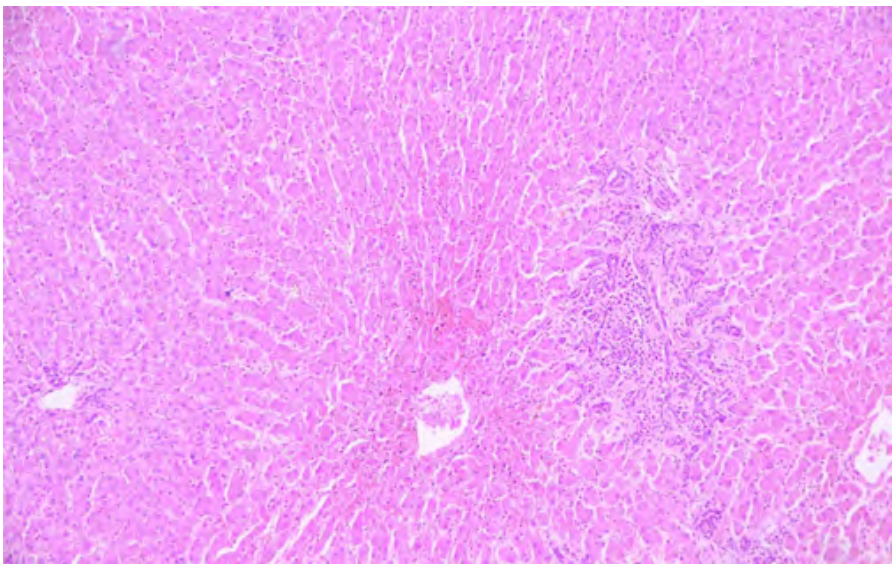

Figure 5. Liver, suppurative cholangitis, cat. This is a significant degree of cholangitis, as can be seen by the presence of hyperplasia of biliary ductules and neutrophilic infiltration of portal areas. However, since inflammation is restricted to portal tracts, the diagnostic features comprise only a small proportion of the overall liver area. Even with a significant degree of inflammation, in a FNA it is still more likely that hepatocytes will be the predominant cell to be sampled rather than the inflammation, precluding a diagnosis of inflammatory liver disease

As for the interpretation of the biopsy, it is not uncommon for surgeons to submit samples in the hope to diagnose specific conditions such as ***inflammatory bowel disease (IBD)*** or ***protein-losing enteropathy (PLE)***. Unfortunately, there are no morphological features that would be specific for either syndrome, and the job of the pathologist is usually to rule- out neoplasia grade the degree of inflammation/mucosal damage, if any, and characterize the predominant inflammatory cell type, and perhaps highlight some common patterns that could be associated with PLE (such as lymphatic distention / lymphangiectasia, crypt abscesses, intraepithelial lymphocytes). It is true that some specific infectious conditions of the intestinal tract may be diagnosed with biopsy (such as histoplasmosis, prototecal colitis, leishmanial enteritis/colitis), but these are exceptionally rare occasions (Figure 9).

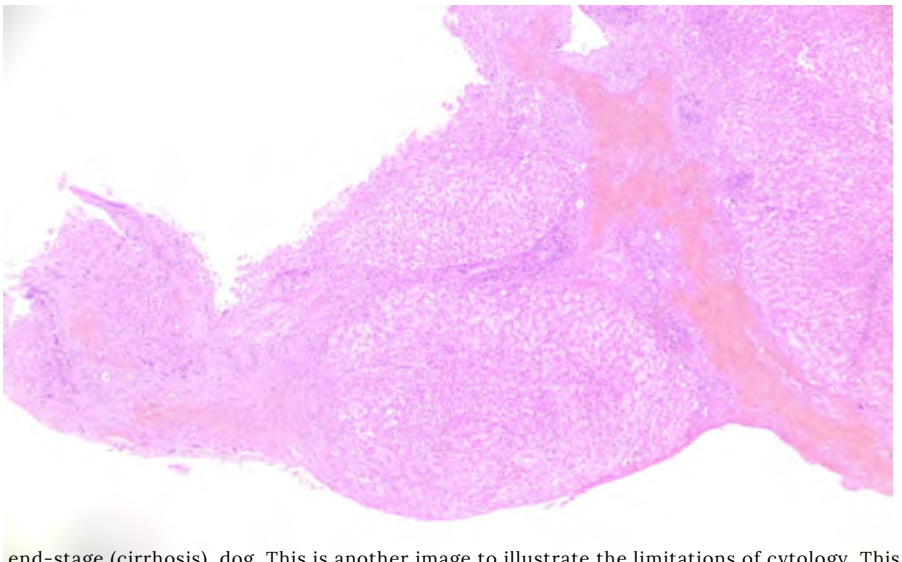

Figure 6. Liver, end-stage (cirrhosis), dog. This is another image to illustrate the limitations of cytology. This is not a highly inflamed liver. However, the diagnosis of end-stage liver disease is made by appreciating the architectural change of the liver parenchyma, with formation of multiple regenerative nodules. In a FNA, hepatocytes would be the predominant cell aspirated, and this particular architectural change could not be appreciated. Also, fibrotic tissue, which also characterizes an end-stage liver, is not readily sampled by FNA, and cannot be relied upon for diagnosis in cytology smears.

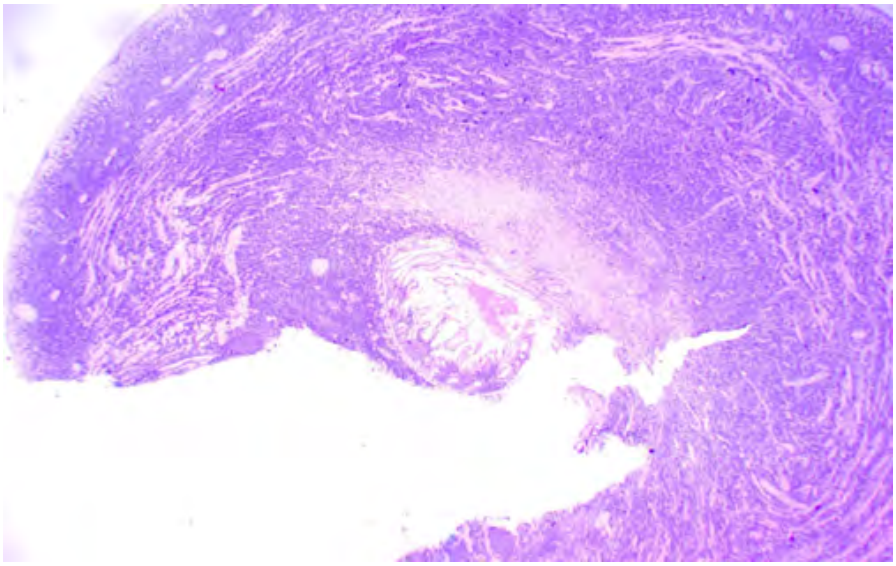

Figure 7. Intestinal lymphoma (cat). This is a section of an intestine from a full-thickness biopsy. There is lymphomatous infiltration throughout all deep layers of the intestine (as evidenced by the dense blue areas, which represent neoplastic cells).

Skin tumours

There are few cutaneous tumours in which surgical excision with margins is essential to prevent tumour recurrence, and in these cases an incisional biopsy may be useful for surgical planning, in cases in which a previous FNA was not successful. These include mainly the common ***mast cell tumours*** and ***soft tissue sarcomas***, but also the not-so-common ***adnexal carcinomas, squamous cell carcinomas,*** among others. Particularly in the cat, a precise diagnosis before surgery is essential in cases of ***injection-site sarcomas***, as these usually require wide surgical margins, and this not uncommonly leads to high post-surgical morbidity. This is also especially important for tumours located on limbs, as this is one of those examples in which a high degree of certainty is usually required before a radical decision such as limb amputation is made. The pet owner would surely not be happy to know that his dog underwent unnecessary limb amputation due to a ***plasmacytoma*** or

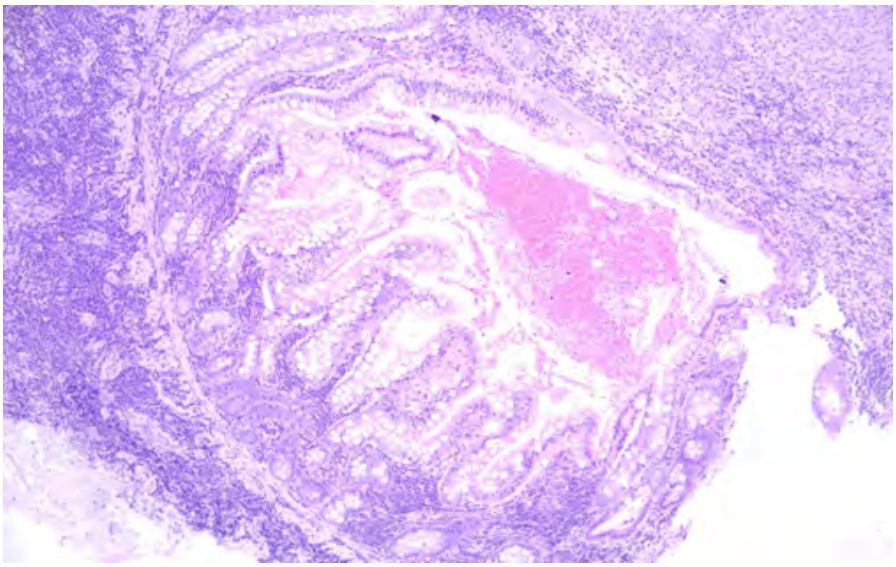

Figure 8. Intestinal lymphoma (cat) - mucosa. This is closer view of a portion of the mucosa of the same case depicted in the previous figure. Although the neoplastic infiltration is unequivocal in deep layers of the intestine, at this area, at the level of mucosal tissue, most pathologists would not diagnose this as a lymphoma. This is to emphasize how neoplastic infiltration may have a variable distribution. When only mucosal tissue is sampled (which is what happens when biopsies are obtained through endoscopy), lesions restricted to deep portions of the intestine might be missed, and this limitation has to be considered when opting for endoscopy.

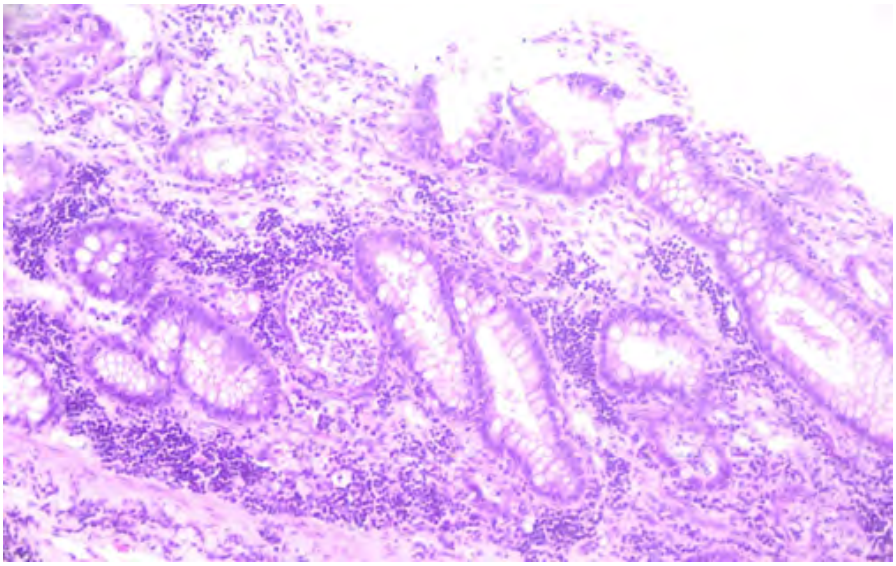

Figure 9. Colon - Tritrichomonas foetus infection (cat). This image is presented as an example of an exception rather than a rule. When taking biopsies from the gastrointestinal tract, one should have realistic expectations with regards to diagnosis. Samples like this one, depicting an infectious agent, are exceedingly rare in practice. More often, biopsies of the gastrointestinal tract describe the type of inflammation (e.g. eosinophilic, lymphoplasmacytic, neutrophilic) and degree of inflammation, if present. It also helps detecting some patterns that may be associated with protein-losing enteropathy (such as crypt abscesses, lymphangiectasia), which is an enteropathy with a worse prognosis. A nosologic diagnosis often has to rely upon ancillary tests and clinical findings. Specific clinical scores (such as the CIBDAI score in dogs) are also useful in determining the clinical severity of the condition and are useful for disease monitoring.

or ***granuloma*** misdiagnosed as ***histiocytic sarcoma*** on previous FNA, or due to a ***fibroadnexal hamartoma*** misdiagnosed as a ***soft tissue sarcoma***. In this context, it is important to appreciate the limitations of FNA when it comes to make these radical decisions.

The limitations of incisional biopsies should also be kept in mind. One of its limitations is related to tumour grading. While grading can be performed in incisional samples, this should be interpreted with some scepticism, especially for soft tissue sarcomas 14, as it is possible that grading will be underestimated or overestimated in small incisional samples. Ideally, grading should be confirmed in the resected specimen.

Bone biopsy
Osteosarcoma (OSA) is the most common bone malignancy in dogs, and this is usually treated by limb amputation. This also requires a high diagnostic accuracy before planning treatment. While FNA samples can at times yield enough cells to allow an unequivocal diagnosis of OSA to be made, it occasionally yields few cells without convincing atypia, precluding a reliable diagnosis.

In cases of a negative FNA, a bone biopsy may be performed, and this is usually done with special equipment such as a Jamshidi needle or Michele trephine, which are specifically designed to collect samples from bone. Ideally, the procedure should be performed after appreciating the radiographic appearance of the lesion. I emphasise that most canine OSAs are central OSA (that is, they arise in the medullary cavity rather than cortex/periosteum). As such, it is important that central areas of the bone are targeted during a biopsy procedure, and this approach has indeed been shown to yield more accurate samples (23). The amount of proliferative tissue present in the cortex and periosteum in a case of canine OSA may be impressive, but not uncommonly these areas correspond only to reactive periosteal proliferations, which commonly occurhappen concurrently with OSA. If only these are sampled, a diagnosis of OSA will not be possible, and additional biopsies will be required (Figures 10, 11 and 12).

Especially because of the possibility of sampling reactive bone rather than neoplastic (and thus rendering a false-negative diagnosis), the accuracies of FNA and incisional biopsy for diagnosing bone tumours have been shown to be comparable, varying from 82% to 92% among different studies (2) (17). Some have recommended performing both techniques at the same time, so as to maximize their diagnostic value (17).

This may be however a matter of personal preference, as both the experience of the surgeon (who will collect the samples) and the pathologist (who will interpret the samples) should be taken into account while deciding the best approach.

Oral tumours
In dogs, the main clinical concern with oral tumours is to diagnose/rule out ***malignant melanoma***, which is the most common oral malignancy in this species. This is usually done initially with an incisional biopsy, and a large tissue sample is preferable. Given that tissue necrosis is a common complication of oral tumours, obtaining a large biopsy often allows an accurate diagnosis of the tumour and provide enough tissue for further analysis (e.g. immunohistochemistry) in case it is deemed necessary (Figures 13 and 14). Considering the differential diagnoses, one common approach is to attempt to remove initially only soft tissue, in a more or less conservative approach. If the lesion turns out to be a ***peripheral odontogenic fibroma*** (POF) or ***gingival hyperplasia***, this may be all that is needed, since they often do not recur, even with incomplete margins. Conversely, if the diagnosis is ***melanoma, squamous cell carcinoma*** or even ***acanthomatous ameloblastoma*** (which is also an infiltrative tumour), further treatment is usually needed depending upon the level of invasion of the neoplasia, as assessed by imaging studies (e.g. mandibulectomy, maxillectomy, radiotherapy). For cats, in the context of mass lesions, the same approach is usually applied, with the main concern being distinguishing the common benign ***granulation tissue*** (also called ***pyogenic granuloma*** - a misnomer that some pathologists avoid) or ***eosinophilic granuloma*** from true tumours such as ***squamous cell carcinoma*** and ***fibrosarcoma***.

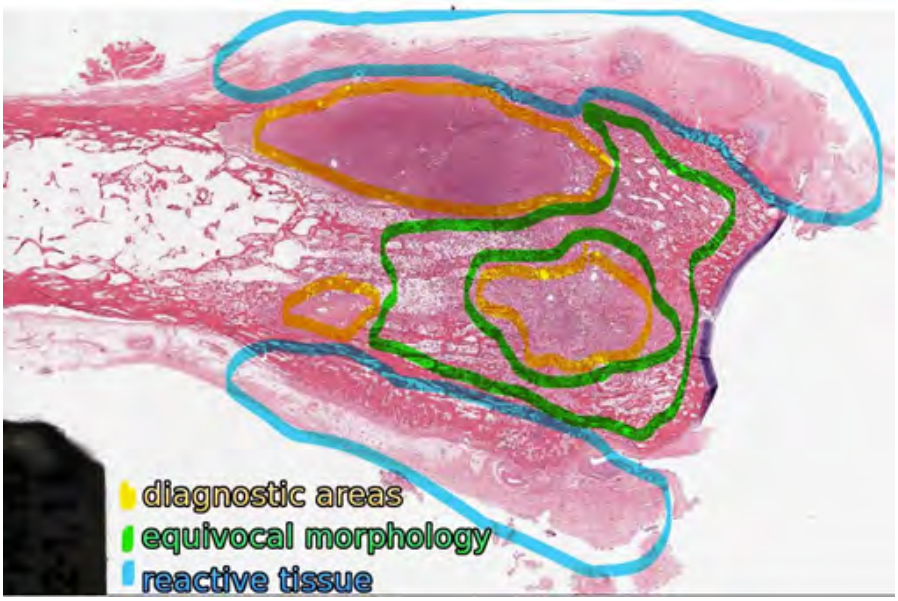

Figure 10. Osteosarcoma (dog). This image highlights the heterogeneity usually seen in canine central OSA. To obtain an adequate sample, the surgeon should aim at collecting tissue from the central areas of bone (highlighted in yellow and green), which are more likely to yield a diagnostic sample (evaluation of radiographic images might help in this respect). Peripheral areas (highlighted in blue) consist of either reactive bone or reactive fibrous tissue, and a diagnosis would not be obtained if only these areas were sampled. Even in the central areas, some portions of the lesion might yield equivocal samples (highlighted in green), which might not be interpreted as unequivocal neoplasia by most pathologists (this also stresses the importance of obtaining multiple samples). Image: Joint Pathology Center. Annotation: Danilo Gouveia Wasques

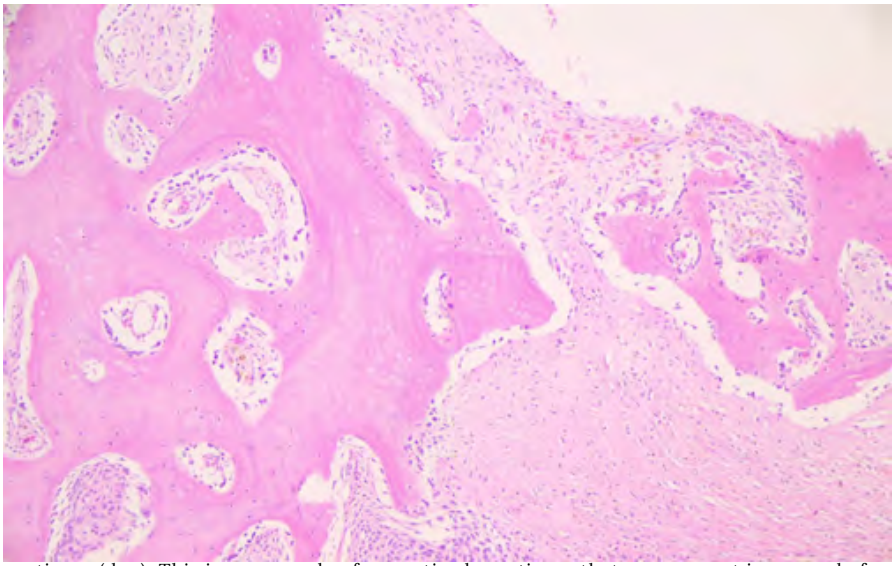

Figure 11. Reactive bone tissue (dog). This is an example of a reactive bone tissue that was present in a sample from a dog harbouring OSA. There is not a dense neoplastic proliferation neither cellular atypia in these cells, and the pathologist thus cannot offer a diagnosis of OSA if only this type of tissue is available (even though the dog does have an OSA).

One particular concern from the pathologist point of view, which applies to biopsies of any organ, but which appears to be more common for attempts at sampling the oral cavity, is the use of electrocautery. The heat produced by this device creates a 'cooked' appearance of the tissue at the borders of the specimen, which may at times render the sample nondiagnostic (Figure 15). It is true that if a large piece of tissue is removed with an electrocautery, these artifacts will be restricted to the borders of the tissue, and the pathologist will often be able to offer a diagnosis (although the evaluation of margins might be compromised). However, if multiple small fragments of the tissue are sampled this way, more often than not the diagnosis will be equivocal, and additional biopsies might be required. To be on the safe side, when planning the surgery, it is better to restrict the use of electrocautery to haemostasis after the incision or punch biopsy.

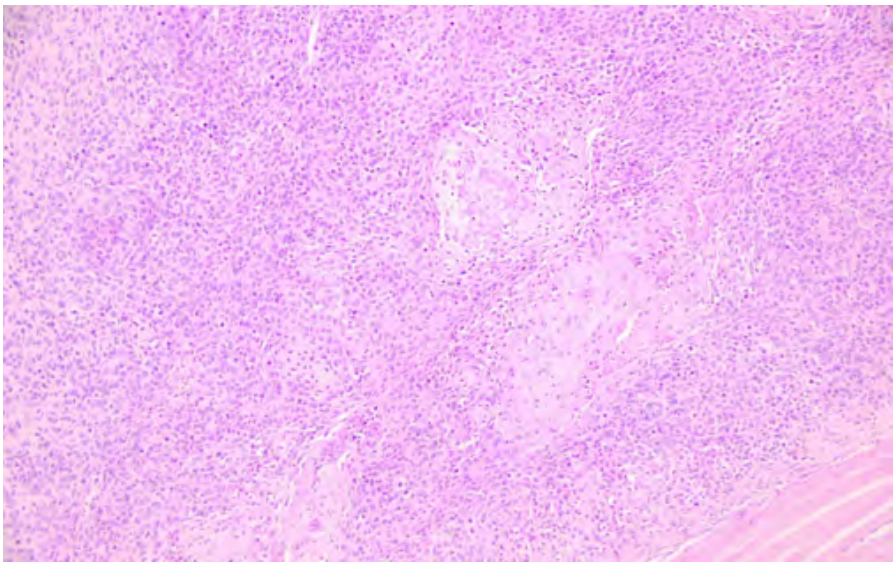

Figure 12. Osteosarcoma (dog). This is from the same sample of the previous figure, but in this area there is an unequivocal neoplastic proliferation of osteoblasts, and a definitive diagnosis can be obtained.

General recommendations for excisional biopsies

After deciding for the excision of a mass, some recommendations apply:

-While ideal, it may be impractical to send large specimens such as amputated limbs, spleens and mammary chains to the lab. If not possible, small portions of the lesion could be sent separately from the whole specimen, which usually allows a diagnosis in these cases. However, the surgeon should retain the surgical specimen in formalin until a report is issued. In this way, if a definitive diagnosis cannot be reached by the pathologist from the small submitted portions, the surgeon could sample more tissue for a more thorough evaluation (this is especially important for splenic samples). Additionally, for proper staging, it is always important to locate and dissect regional lymph nodes for evaluation. Finally, if there is a concern regarding surgical margins, these should also be sent for evaluation (and properly labelled, so as the pathologist can be certain about which tissue corresponds to the margins).

Surgical margins

Assessment of margins is often even more important than the diagnosis ***per se***, and this is an important part of the job of the surgical pathologist. As a general rule, if evaluation of margins is anticipated, the resected specimen should not be incised after removal from the patient. This is because there would be a risk that the pathologist would interpret a post-surgical incision as a true surgical margin, and a false positive dirty margin could be diagnosed. This could lead to unnecessary additional surgery. If incision of the specimen is inevitable (such as in large samples - see previously), the surgical margins should be properly labelled/identified. This would be ideally done with ink, but can also be satisfactorily done with sutures (provided this is properly referenced in the submission form). If surgical resection is performed in multiple steps (such as in debulking surgery or widening of deep margin), ideally the tissue that was last removed from the patient should be labelled separately for margin evaluation.

Figure 13. Melanoma (dog). This is a low-power magnification of a sample of oral melanoma. Most of what is presented in this image consists of necrotic tissue, and a diagnosis cannot be made based on this. This is to illustrate how commonly oral tumours (especially malignant ones) may undergo necrosis, and due to this, if only small pieces of tissue are sampled, a diagnosis might not be possible.

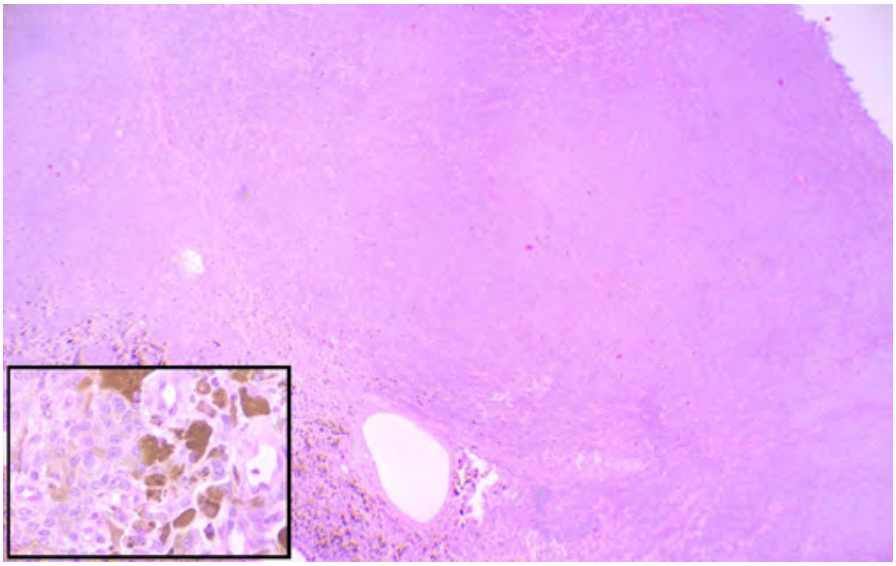

Figure 14. Melanoma (dog). This is more cellular and diagnostic area of the same tumour depicted in the previous image. Inset - higher magnification: pigmented neoplastic cells are clearly evident.

Significance of dirty margins

We routinely report on surgical margins from all specimens, even for benign tumours. But it should be emphasized that a diagnosis of dirty margins (or abnormal tissue to the margins, incomplete margins) does not necessarily indicate the need for additional surgery or local therapy. Although this is true for malignant/aggressive tumours (such as high grade mast cell tumours, soft tissue sarcomas and carcinomas (18), benign tumours with dirty margins usually do not need additional treatment.

The distinction between benign and malignant may be however blurry for some tumours. For instance, low grade mast cell tumours might not recur even with incomplete margins if an attempt was made to remove the entire mass. While it is important to include wide margin resection while planning surgical excision of mast cell tumours, this may not be feasible in some cases (e.g. tumours located at the extremities of limbs). Some authors have recommended that, for low-grade mast cell tumours with

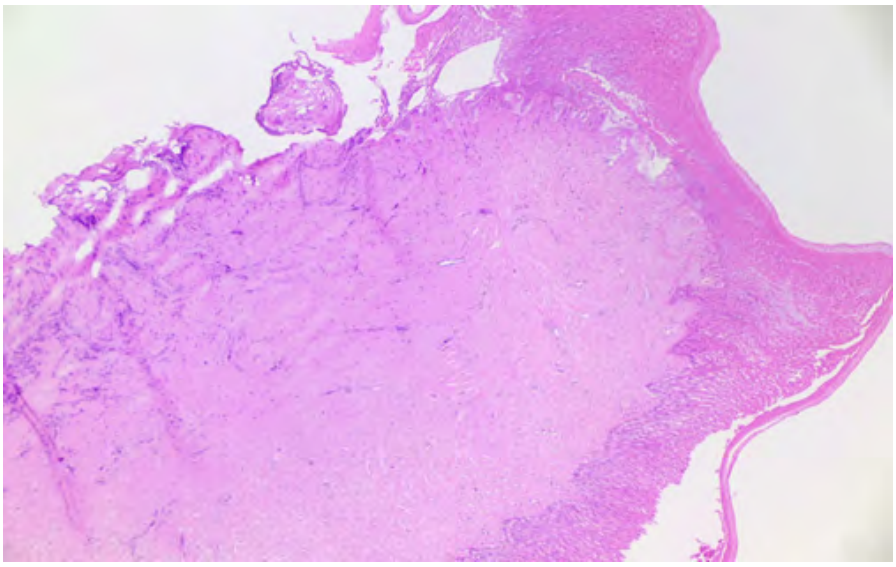

Figure 15. Oral mucosa (dog). This section has a coagulated ('cooked') appearance at the edges of the specimen. This is caused by the heat produced by the electrocautery, and may at times preclude a proper diagnosis.

incomplete margins, it is worth performing additional immunohistochemistry for proliferative markers. In case it proves to be a tumour with low proliferative activity, additional local treatment might not be needed, as the disease-free intervals from this subset of tumours with complete or incomplete margins are similar (i.e. they are unlikely to recur) (19) (20).

Another example of a cloudy distinction between benign and malignant behaviour is that of ***perivascular wall tumours (PWT)***, which is also called ***haemangiopericytoma*** or simply ***spindle cell tumour*** by some pathologists. These are more often a low grade mesenchymal neoplasia, with a negligible chance of metastasis. However, although a wide surgical excision is often desirable, this may also be difficult to be achieved for tumours located on distal limbs (which is a common location for these neoplasms). In these cases, some authors suggest that a more conservative approach may be employed, as the chance of recurrence is not high even for tumours with close or dirty margins, and when it does happen it is usually after a long period after the excision (24% to 53% of cases recurring in 3 years after excision) (1) (9) (21). The same point of view is not shared by other authors, however, who advocate a more aggressive initial surgical excision whenever possible 9. At this point, this may be a matter of personal preference and experience, and a frank discussion with the pet owner to consider the benefits and drawbacks of each approach is important.

Bone specimens

Histological diagnosis is usually made quickly, but samples from bone usually require more time to be processed.
This is because they need to be decalcified prior to sectioning in the microtome. As a rule, we usually process soft tissues beforehand and issue a preliminary report before processing the bone, but some delay in the definitive report should be anticipated in these cases.

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