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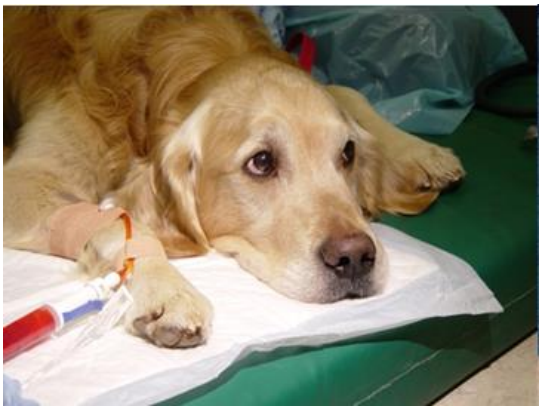
Treating aggressive sarcomas & carcinomas in Small Animal Practice Mini Series

Session One: Introduction & all about soft tissue masses (including feline injection site sarcoma)



Treating Aggressive Sarcomas and Carcinomas
Study Notes: Session 1

1a: Cancer Biology, Pathology & Treatments

Cancer treatment in dogs and cats has historically been neglected on the assumption that it would have unacceptable side effects and implications for animal welfare. The opposite is in fact true – since our pets don't have decades of natural life expectancy, a very acceptable treatment outcome can be achieved by treating the cancer like any other a chronic disease, seeking to slow its' development and control clinical signs allowing the animal to maintain a their previous quality of life, rather than trying to annihilate all the cancer tissue. Furthermore, refusal to entertain treatment or palliation of a disease with an "I'm sorry there's nothing we can do" approach will often result in clients taking the care of their pet into their own hands, with treatable pain, nausea and other clinical signs going untreated.

In this first session we will explore some basic principles of cancer diagnosis and treatment before proceeding to look at soft tissue sarcomas, in particular at the devastating feline injection site sarcoma in more detail.

Cancer cells are cells that have escaped the body's control and are dividing and growing in an autonomous fashion. Their behaviour is analogous to a population of bacteria in an infected wound – subject only to natural selection and survival of the fittest. Thus, we can explain local invasion and metastasis from the evolution of motile cancer cells which can escape a hypoxic and nutrient-deplete tumour centre. Similarly we can explain the evolution of drug resistance in a tumour, since use of any drug (or any intervention we give!) will apply a selection-pressure.

The pathologist's report will describe how well-differentiated cancer cells are (how much they resemble their benign counterparts); the implication being that the most aggressive cancers will have minimal (or no) similarity with their cell of origin, focusing only on cell division at the detriment of expressing the normal genes expected of that tissue. The most aggressive cancers will also express genes at random and so you can see cellular features which are not considered normal for the tissue of origin. The pathologist's report will also comment on the number of mitotic figures observed (as a proxy for the speed of growth of the cells, thus allowing us to predict rate of development of disease and choose appropriate treatments), the interaction with the surroundings (an indication of necessary surgical margins) and whether emboli of cells invade lymphatics or blood vessels (a suggestion of metastatic potential). Finally the pathologist will determine the histogenesis of the tumour, allowing us to better predict the likely behaviour and appropriate treatment. However, where determination of histogenesis is not possible, we can see that much of the tumour's behaviour and treatment can be predicted from a first-principles understanding of the pathology report.

Surgery has been the primary treatment modality for cancers for hundreds of years. Care has to be taken however to resect the tumour and all of its' "root network," and a CT scan be very useful in identifying occult extension of the tumour. Surgical oncology is a "Marmite-like" subject among surgery specialists; many leading surgery specialists do not like performing aggressive resections and so, in the face of a very aggressive tumour, it will pay dividends to find a surgeon who is enthusiastic about the necessary procedure. When performing a tumour resection, I would encourage the surgeon to mark or ink the margins, and to indicate

(for example with the use of a staple or suture) where areas of concern for incomplete excision lie, the pathologist can inspect these areas for incomplete excision in some detail.

Radiation therapy (RT) is the second oldest treatment for cancer, and is also a local therapy (treating one site in the body, rather than acting systemically). RT seeks to damage cellular DNA, such that when the cell tries to divide it will be unable to replicate its' genetic material and undergo a "mitotic death." The sensitivity of a cancer to radiation depends on the tissue in question (some tissues are much better able to repair radiation damage than others) and the bulk of the tumour. The simplest way of improving radiation sensitivity is to surgically "debulk" the tumour since this will increase the proportion of cells which are actively-dividing (radio-sensitive), but chemotherapy drugs (for example carboplatin) can also be used as "radiation-sensitizers") since these drugs will potentiate DNA damage and impede the action of DNA-repair mechanisms.

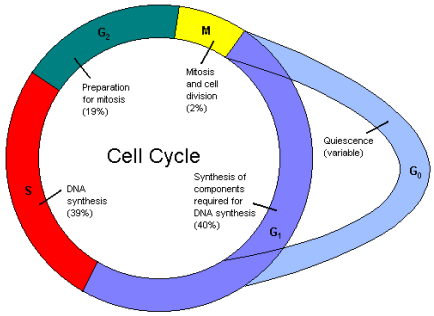


Figure 1: The cell cycle

There are two fundamental types of RT. Definitive protocols aim to cure the lesion, and involve hyperfractionation (giving lots of small doses of radiation; typically 12-20 doses, often on at least three days a week for several weeks consecutively). Definitive treatments can cause acute side effects (for example mucositis of mucous membranes and dry desquamation of skin). These adverse effects limit the dose of radiation prescribed but rapidly resolve when treatment ceases. Palliative protocols aim to control pain and inflammation associated with the tumour and involve hypofractionation (typically giving 2-4 large doses of radiation). Such protocols rarely cause acute problems however they have a much greater risk of delayed radiation toxicity, usually occurring at least 6 months after RT is administered (often at least 1 year later). Delayed toxicities typically involve necrosis of late-responding tissues (for example bone, or nervous tissue), or fibrosis (for example strictures in the gastrointestinal tract); these adverse events are usually catastrophic and often result in euthanasia of the animal concerned. Despite the potentially-devastating nature of late toxicities however, palliative radiation protocols are very effective means of pain control, and can be very safely used in true "hospice-care" cases where the life-expectancy of the animal is less than 6 months.

Cytotoxic chemotherapy is a relatively recent form of cancer therapy, developed principally for lymphoid malignancies and is the legacy of accidents and crude experiments with chemical warfare in the first half of the twentieth century. Cytotoxic drugs work similarly to RT in that they damage DNA such that the cell is replicate genetic material, or sometimes interfere with the machinery of cell division directly; they are divided into two groups, the cell-cycle specific drugs (where the only susceptible cells are those at a specific point in the cell cycle), or cell-cycle non-specific drugs (where all cells are susceptible but the lethal effect is only realised when the cell tries to divide). Traditionally, cytotoxic drugs are given at the "maximum tolerated dose" (MTD), and repeated as soon as possible after healthy

tissues have recovered. This kind of dosing works best where a large proportion of the cancer cells are in the cell cycle, thus is most effective in lymphoid malignancies in dogs and cats. Sarcomas and carcinomas (mostly) have a much smaller proportion of cells within the cell cycle and so their response is typically much less dramatic (although notable exceptions exist, as we will learn later!). Similar to RT, the chemo-responsiveness of a tumour can be improved by surgically debulking so that a greater proportion of remaining cancer cells will enter the cell-cycle and become sensitive to drugs.

Since MTD chemotherapy dosing was not curative for a number of important human cancers, attention was given to other forms of cancer treatment, and targeting angiogenesis has found considerable favour. In targeting angiogenesis we are targeting the benign host cells upon which the tumour relies; thus we temporarily avoid the problem of cancer cells evolving drug resistance, and we can dose chemotherapy in a fashion which is much more sparing to host tissue, and suitable for long-term use without significant adverse effects. The most important example of anti-angiogenic therapy in veterinary medicine is metronomic chemotherapy which has been shown to be effective in some situations where MTD chemotherapy is ineffective. Metronomic chemotherapy has subsequently been shown to control the activity of T-regulatory lymphocytes (thus allowing a stronger anti-cancer immune response) as well as the anti-angiogenic effect. Some of the tyrosine kinase inhibitor drugs can also be used, off-license, to inhibit important cell-signalling pathways involved in angiogenesis (for example toceranib, "Palladia" will inhibit the receptor for vascular endothelial growth factor, VEGF, and masitinib, "Masivet" will inhibit the receptor for platelet-derived growth factor, PDGF, both important angiogenic ligands.

Recent advances in the understanding of cell biology has facilitated the development of toceranib, masitinib and many other small molecule inhibitors (SMIs). Cancers usually involve the constitutive, inappropriate activation of molecular pathways which promote constant cell division and growth, often also rendering the cells insensitive to homeostatic mechanisms to control these harmful processes. These molecular pathways are quite pleotrophic; inhibition of one key molecule in the pathway will abrogate many downstream effects. Many (but not all) of the molecular pathways inhibited in this way involve tyrosine kinase receptors. For example, the mutated, c-KIT protein (a tyrosine kinase) constitutively activates downstream pathways (in the absence of a ligand) in canine mast cell tumours, and inhibition of this protein with toceranib or masitinib represents the best example of small molecule inhibition in veterinary medicine. Many more similar drugs are likely to follow in the future. As attractive as small molecule inhibitors may appear however, they are no panacea; another feature of cancers is the concept of redundancy –the same cellular process can be achieved through several different molecular pathways and inhibition of just one of these will often produce a transient reduction in cancer burden before the cells evolve resistance, characterised by increased activity of other pathways. Thus, most human use of SMIs involves combination therapy – both with other SMIs and cytotoxic chemotherapy. In veterinary medicine, we are only just beginning to find out how Much work is yet to be done before we can cure a lot of cancers.

1b: Soft Tissue Masses and Soft Tissue Sarcomas

The small animal veterinarian is constantly shown lumps and bumps in the skin and soft tissues of cats and dogs, often with an owner seeking reassurance that they are nothing to worry about. It is certainly true that a mass that appears very aggressive (poorly-demarcated,

painful, red, heterogenous etc.), is usually very aggressive, the opposite is sadly not true. A "non-frightening" mass could equally be a malignant tumour as it could a benign one. Thus a pro-active approach to soft tissue masses is required, and I would recommend fine needle aspiration (FNA) in the first instance. "Needle-only" FNA technique is a reliable test for lipomas, mast cell tumours and a number of epithelial tumours. Sarcomas don't exfoliate readily however if a needle-free technique doesn't give a diagnostic cell yield, then repeating FNA with several mls of negative pressure will often provide a diagnostic sample. It is good practice to check cytology in-house, and preferably at the time. Checking cytology in-house will avoid wasting pathology fees on blood-only, or karyolitic smears, and it will also help to identify a neoplastic lesion from an inflammatory lesion (in the case of septic inflammation it is useful to know that antibiotics are justified as early as possible after presentation). A biopsy of the tumour is often unnecessary if care and attention is paid to the FNA technique. Detail of FNA technique is provided in an appendix.

Excisional biopsy of mass, as tempting as it can be for both owners and vets, is to be discouraged. As much as owners may "just want the lump removed," they usually don't understand that excisional biopsy will distort the tissue architecture and break up protective fascial planes, facilitating the tumour's migration and making a second surgery harder. In aggressive tumours (as we will see later!) there is clear and categoric evidence that excisional biopsy will undermine the animal's prognosis. In some cases this will mean the difference between life and death!

Soft tissue sarcomas (STS) are cancers of mesenchymal cells of the soft tissues. There are thus many different histological types, however most of them will behave in the same way and identification of the precise histological origin is not necessary. A couple of specific subtypes for example the haemangiosarcoma and histiocytic sarcoma have different behaviours and will be covered separately. Diagnosis an STS can be readily accomplished using FNA technique involving negative pressure in most cases.

STS are histologically divided into 3 grades, based on the mitotic index, presence of necrosis and the cellular differentiation. All 3 grades have clinical relevance; the recurrence rates of tumours after incomplete excision is different for each grade (approximately 7% for low grade, 34% for intermediate grade and 75% for high grade), and the risk of metastasis differs for high grade tumours (40-44%) versus the lower grades (less than 15%). Most STS encountered in first-opinion practice are of low and intermediate grade and given the low metastatic rate of these tumours, most STS are thus considered local problems, often curable with adequate local control.

Surgery is the first treatment to consider with all soft tissue sarcomas. Owing to their marked local invasion, margins of 3cm horizontally and one fascial plane deep are recommended, nevertheless in one study of 104 STS managed in primary-care practice, 3cm margins were achieved in fewer than 10% of patients and the recurrence rate was only 28%. STS are relatively insensitive to radiation so RT for a gross STS has limited effect. RT is best used to treat the scar of an incomplete surgical resection – it is a very effective treatment when used this way. Metronomic chemotherapy has also been shown to control STS recurrence after incomplete excision. In the case of an incomplete resection however, one must first consider the grade of the tumour since an MTD chemotherapy protocol will be indicated for a high grade STS in all instances, owing to its' metastatic rate, and this treatment will help to control recurrence as well as metastasis. If a low or intermediate grade STS is incompletely-excised then the first question should be "is a revision surgery possible?" before considering RT and

then metronomic chemotherapy in that order. When considering a pathology report which describes a tumour as completely-excised it is necessary to also consider how the pathologist assessed the margins for completeness of excision; where absolute certainty of completeness of excision is needed the "shaved margins" technique should be requested.

In the case of a high-grade STS local control should still be used since the primary tumour is often associated with significant local damage and pain. The metastatic rate of these tumours also dictates that chemotherapy is used to control development of metastatic disease and the standard-of-care treatment would be a doxorubicin-based protocol involving intravenous doxorubicin injections every 3 weeks. A cheap and easy way to intensify this protocol involves giving an oral cyclophosphamide dose in between each doxorubicin injection (the "AC" protocol, see haemangiosarcoma lecture) but no clinical benefit of adding cyclophosphamide has been shown.

The feline injection site sarcoma (FISS) is an extreme example of a STS, and a good illustration of the challenge of dealing with a high-grade STS in any species. A FISS is formed due to the propensity of the feline fibroblast to mutate and lose control of cell division under the climate of chronic inflammation; it can therefore form at any site of chronic trauma, not just an injection. FISS have now been reported around microchips, lufenuron implants, insulin injection sites and long-acting steroid injections, as well as sites of trauma and many other causes. The histology of FISS can vary, sometimes suggesting fibrosarcoma, other times myxosarcoma or undifferentiated sarcoma. Different grades are possible too, but this often has little clinical relevance. *I would never trust any sarcoma found at any site of injection or previous trauma in a cat,* especially when the histology report describes the neoplasm being surrounded by inflammation. Where a suspicion of FISS arises I would recommend talking to an oncology specialist at the earliest possible convenience.

Although the metastatic rate of FISS is modest (in the order of 25%), most cats are euthanased within a year of diagnosis of the tumour due to the intractability of the tumour's invasion and the consequent morbidity. In surgically-resecting these tumours, margins of 5cm horizontally and 2 fascial planes deep are recommended. Although the principles of removing a FISS are the same as for any other STS, the resection of such a tumour has to be so bold that referral to an experienced surgery specialist, with an interest in onco-surgery should be offered at the earliest possible point in the case. Prompt intervention with a very aggressive surgery is frankly the only way we can save these cats. A more conservative resection (for example with 3cm margins and one fascial plane) or an excisional biopsy of these tumours will fuel the fire, creating inflammation from which the tumour can "feed," it will destroy fascial barriers preventing spread of the tumour and a FISS will not forgive such an endeavour; it has been shown beyond question that the need for a second surgery will correspond to a poor prognosis. Similarly removals of FISS by non-specialist surgeons correlate with a survival time of less than half of that if the surgery is done by a specialist. Practitioners in the front-line of veterinary practice therefore hold the prognosis of these cats in the palm of their hands!

Nearly all deaths from FISS are related to the local disease, and metastatic disease, if it occurs, is often clinically-irrelevant. Metastatic disease is much more common in cases of a high-grade FISS compared to other grades, so it is logical to offer chemotherapy for all high-grade cases, but as for other high-grade STS, surgery is still the primary therapeutic consideration. Adjunctive RT should be offered for incompletely-excised FISS (regardless of

the grade); pictures of the tumour pre-surgery and implantation of radiodense material (for example surgical staples) to mark the extent of the surgical resection are essential to accurately deliver such a therapy. In the case of large FISS, some radiation oncologists prefer to radiate the tumour *before* surgery (the rationale being that the target of the radiation is smaller and so a higher RT dose can be focussed on the cancer cells, risking a smaller area of "collateral damage" to healthy tissue), so it is helpful to discuss with a radiation oncologist before surgery occurs.

Chemotherapy (doxorubicin-based) in the gross tumour setting has an approximately 40-50% rate of response, with responses lasting for no more than a few months. Nevertheless, chemotherapy is often used as part of a multi-modal treatment for these cats, particularly if excision is incomplete and RT is not possible. There is also an argument for chemotherapy due to the genesis of these malignancies; since the tumour is borne from a state of chronic inflammation, many cases of tumour recurrence may be due to new tumours forming de novo, than regrowth from cancer cells remaining at the surgery site. This may be one reason why the best results in the treatment of FISS typically involve multimodal therapy. Often a combination of radical surgery, RT and MTD chemotherapy are used and the total expenditure by the client is often in 5 figures.

Numerous other medical therapies have been trialled for FISS with limited success. Results from the use of masitinib and toceranib in particular have been disappointing. Electrochemotherapy and immunotherapy with exogenous feline interleukin-2 have shown initial promise, however more studies are required to verify this. Ultimately, there remains no good substitute for aggressive, often multi-modal therapy when dealing with a FISS.

Finally, all small animal practitioners should be familiar with the histiocytic sarcoma (HS). The HS is not a mesenchymal cancer, it is a round cell tumour (from monocytes, dendritic cells or macrophages) and has a lot in common with lymphoma. HS is sporadically seen in first-opinion practice and although there is a strong breed-linkage (retriever breeds, Schnauzers, Bernese Mountain Dogs etc.), it can occur in any dog.

Three distinct forms of HS exist. Localised HS (LHS) is a common condition in Flatcoated retrievers, and often involves tumours associated with the appendicular skeleton, presenting with lameness and swelling. These lesions are very radio-sensitive however and so amputation is not needed – a hypofractionated radiation course (cheap, often 3-4 doses at weekly intervals) will put the local tumour into remission and resolve the dogs' lameness. LHS remains a metastatic disease however and so medical therapy is indicated to control development of metastasis. Lomustine is considered the treatment of choice, given orally, every 3 weeks, monitoring for haematological and hepatic toxicity before each dose. LHS can also occur as solitary masses (usually in lungs or mediastinum) and after surgical removal the treatment and prognosis is similar. The expected survival time for LHS is in the order of 10-18 months.

Disseminated HS (DHS) is a much more aggressive disease, often involving the viscera, and metastasizing rampantly. Metastases to all tissues are possible (for example DHS metastasis in the CNS, or infiltration of the bone marrow are not uncommon) but lungs, lymph nodes and abdominal viscera are most common. Surgery is only indicated as a first-aid measure or to palliate severe discomfort; medical therapy with lomustine is the standard-of care. The response rate to lomustine is in the order of 50%; responders can expect a survival time in the order of 6 months and non-responders 2-3 months. In cases which don't respond to

lomustine, a small amount of success has been reported with vinorelbine and there are anecdotes of modest success with tyrosine kinase inhibitor drugs. Anaemia, thrombocytopaenia and hypoalbuminaemia are considered negative prognostic indicators for DHS.

Finally, haemophagocytic HS (HHS) is the third variant of HS, and is characterised by an intractable anaemia and often thrombocytopaenia. These cases are often misdiagnosed as IMHA cases initially, and failure to respond to treatment prompts search for an underlying cause. Infiltration of neoplastic cells in bone marrow, liver and spleen is common, and erythrophagocytosis by the cancer cells is often an obvious feature. No effective treatment for this variant of HS has been described and survival times are in the order of days to weeks.

Reference Books for this Course:

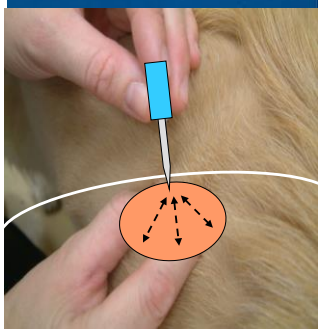
- Withrow, Vail, Page; "Withrow & Macewen's Small Animal Clinical Oncology;" 5th Edition; Elsevier.
- Raskin, Meyer; "Canine and Feline Cytology: A Color Atlas and Interpretation Guide;" 3rd Edition; Elsevier

Further Reading for Session 1:

- Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell 100, 57–70.
- Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: The Next Generation. Cell 144, 646–674.
- Biller, B. (2014). "Metronomic chemotherapy in veterinary patients with cancer: rethinking the targets and strategies of chemotherapy." Vet Clin North Am Small Anim Pract 44(5): 817-829.
- London, C.A (2014). "Small molecule inhibitors in veterinary oncology practice." Vet Clin North Am Small Anim Pract 44(5): 893-908
- London, C.A et.al (2012). "Preliminary evidence for biologic activity of toceranib phosphate (Palladia) in solid tumours." Veterinary Comparative oncology 10(3): 194-205
- Elmslie RE, Glawe P, Dow SW. "Metronomic therapy with cyclophosphamide and piroxicam effectively delays tumor recurrence in dogs with incompletely resected soft tissue sarcomas." J Vet Intern Med. 2008 Nov-Dec;22(6):1373-9.
- doi: 10.1111/j.1939-1676.2011.0753.x. Epub 2011 Jul 7.
- Burton JH[1], Mitchell L, Thamm DH, Dow SW, Biller BJ. "Low-dose cyclophosphamide selectively decreases regulatory T cells and inhibits angiogenesis in dogs with soft tissue sarcoma." J Vet Intern Med. 2011 Jul-Aug;25(4):920-6.
- Mitchell L[1], Thamm DH, Biller BJ. "Clinical and immunomodulatory effects of toceranib combined with low-dose cyclophosphamide in dogs with cancer." J Vet Intern Med. 2012 Mar-Apr;26(2):355-62.

Appendix:

FNAs; my approach

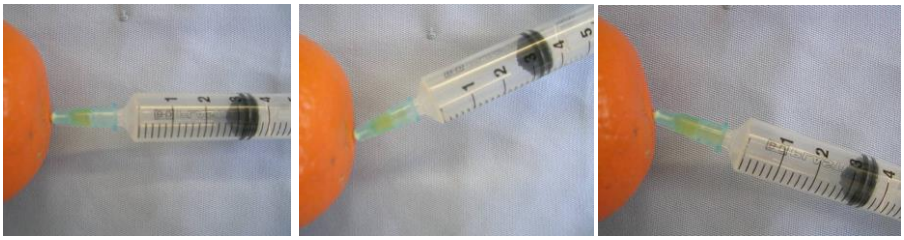


"Needle-only" first…..

- Needle 19-25G; 23G common
- Syringe
- Slides
- Immobilise mass with one hand
- Used other hand to sample lesion
- Good for superficial masses and LNs
- 23G needle, redirect several times, without exiting skin

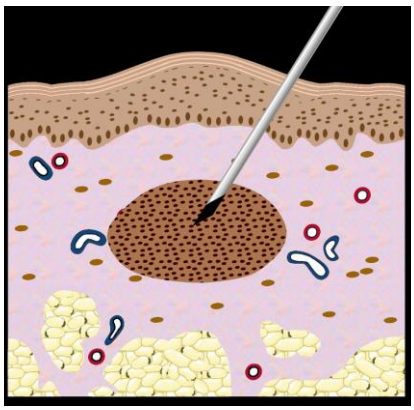
"Suction technique" if needle-only doesn't yield cells…..

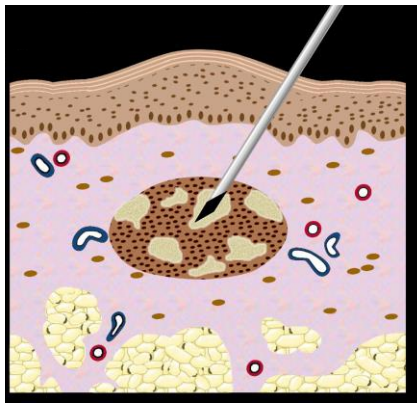
- Needle 19-25G; 23G common
- 5-10mls negative pressure maintained whilst redirecting needle several times
- *Release negative pressure before withdrawing through skin



FNA- tips

- Avoid sampling cystic/necrotic areas
- US can help in some circumstances





- **Always** get several samples and check quality!

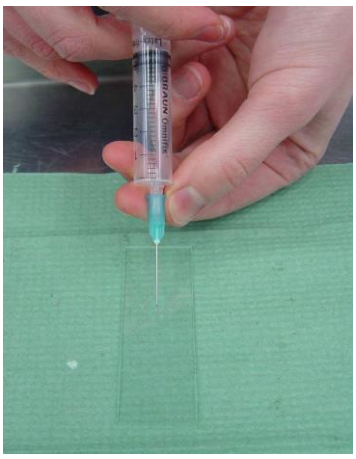
  There is nothing wrong with repeating an FNA until a representative sample is obtained

  - Sampling technique is extremely important for cytologic diagnostic accuracy
  - If your first sample was non-diagnostic, try to improve your technique the second time!

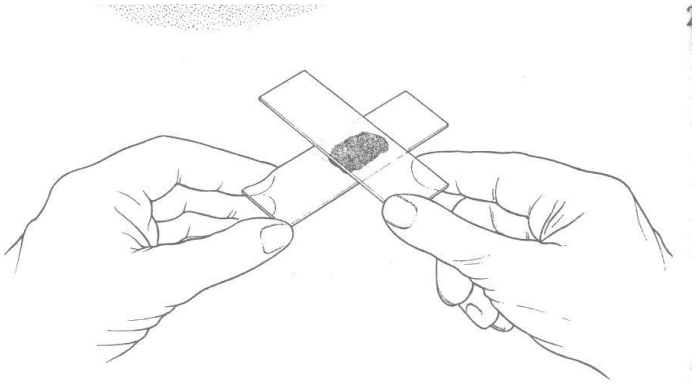
- Avoid excessive suction
  - Causes haemodilution and cell damage
- Do not use >21G needle
  - You will get a sample that is too thick!
- Be quick in spreading! it will improve sample quality

Making smears...

- Once sample is obtained within needle
- Attach an air-filled syringe
- Position needle close to microscope slide
- Rapidly expel contents
- Make smear…
  - "Squash" preparation



“Squash” preparation



“Squash” preparation tips

- Don’t “squash” too hard!!
- BE GENTLE, or the cells will rupture