PEARLS OF ONCOLOGY

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FINE NEEDLE ASPIRATION TECHNIQUES

Sample collection and preparation for cytology is simple and requires little equipment. Needles (25, 22, 20, or 19 gauge), 6 cc syringes, clean glass slides, and occasionally a scalpel blade for scraping are all that is needed. In vivo collection technique can be performed with a needle alone or with a syringe attached to the needle. Before obtaining the sample, make sure the slides are set out (at least 4-6) and the syringe is ready. Stabilize the mass in your non-dominant hand. For the needle alone technique, direct the needle into the mass; withdraw (yet stay within skin) and push back into the mass several times rapidly. Draw several cc air into the syringe, connect it to needle and expel needle contents onto a slide (make sure the bevel is down and the contents come out near the frosted end). For the needle plus syringe procedure, connect the needle and syringe, direct the needle into the mass, draw back plunger and release several times, with pressure released withdraw the needle from mass, disconnect the needle and draw several cc air into syringe, reconnect and expel needle contents onto the slide.

Slide preparation is key for good cytology results. The samples must be smeared out quickly to prevent clotting and need to be in a thin layer for best cellular evaluation. Even if only a small dot of sample is expelled onto the slide, smearing it is important. Cells that dry in a little round "puddle" of fluid maintain more of a 3-dimensional appearance, do not spread out their cytoplasm, and are difficult to assess. After the contents are expelled onto a slide, place a second slide onto the first cross-wise to the first slide) and pick up a SMALL amount of material. Pick up a third slide, gently place the second slide across the third slide to squash the sample flat and smear the sample by pulling the second slide away from the frosted edge of the third slide while maintaining slide contact with gentle pressure. The second slide needs to stay FLAT on the third slide (no edge to catch cells and drag them). Now, the second slide can be used again to pick up another small bit of sample from slide 1, and can be smeared onto a 4th slide, etc. When just a small amount of sample remains on slide 1, use slide 2 to smear it as well. Most aspirates will yield 3-6 good slides, allowing for in-house staining and evaluation of one slide while leaving several unstained for laboratory evaluation. Also, by making multiple smears, the samples are more thinly spread out which improves diagnostic ability. This is particularly helpful with very cellular aspirates.

CYTOLOGY SCREENING

Once the slide is stained it is ready for microscopic examination. Scan the slide on low power (10x) first to get an overall feel for the cellularity of the sample and then go to higher power to evaluate the cell types and individual cytologic features of the cells. The two most important questions as a practitioner to answer when looking at a slide are 1. Did I hit the intended tissue? (salivary gland vs. lymph node, blood vs. bone marrow) and 2. Is it a good sample? (cells not all ruptured, enough cells for evaluation). After assessing these points, evaluate if the cells are from normal tissue. Note if inflammation (particularly neutrophils) is present. If so, caution must be used in interpreting other cells on the slide. Inflammation can induce cells to look "malignant" when they are actually just reactive, especially macrophages. Next ask if there are cells in abnormal places (non-lymphoid cells in lymph node, non-hepatic cells in liver). Finally, note the predominant cell type.

1. Epithelial: cluster, even away from the large groups, may see 3 dimensional structures, tend to be ruptures away from the clusters
2. Mesenchymal: solitary, may appear as a cluster but will be intact and individual away from group. Wispy, trailing cytoplasm with hard to define margins
3. Round: exfoliate well, often very cellular but do not form 3 dimensional structures. Distinct cell margins, round shape (although may be angular)
    a. Round cell tumors include: lymphoma, plasma cell tumor, mast cell tumor, transmissible venereal tumor, histiocytomas, melanoma

If epithelial or mesenchymal, the next step is to assess for features of malignancy. Both of these types may require a biopsy to confirm malignancy. The malignancy of round cell tumors is dictated by their location more than their cytologic appearance (for e.g., cutaneous plasma cell tumors are mainly benign, with GI one are malignant).

CORE COMMUNICATION SKILLS

Non-verbal communication

A large percentage of all communication between individuals is nonverbal and generally unintentional. Thus, information that cannot be hidden is being exchanged at all times – both from the client to the clinician, and vice versa. The nonverbal aspects of communication are like a poker player's "tell" – they give away what a client may be thinking or feeling, possibly contrary to what they are saying verbally. Emotions are much more often communicated non-verbally than verbally. For example, we know when someone is mad because they raise the volume of their voice, have a different tone, change their posture, etc, not because they say "I'm so mad".

Mixed messages occur when the verbal and nonverbal messages disagree. In these situations, the nonverbal message is more accurate and needs to be detected and addressed. A common experience is asking a client if they understand, and the client hesitantly says "yes", often without meeting the clinician's eyes. The non-verbals clearly say the client does NOT understand, and paying attention to that fact and trying to clarify things at that time will often save misunderstanding and upset in the future.

Another benefit to becoming aware of and following nonverbal clues is that they take up no extra time during an interaction. They are going on constantly during all interactions, and one needs to only consciously note them (and perhaps comment on them, see below) to help guide the discussion in a beneficial manner.

Empathy

Empathy does not mean one feels what the client feels, but rather that one can try to imagine what the client has or is experiencing. It is the proverbial "walking in someone else's shoes", and a key point is not only to imagine that experience but to SHOW the client that understanding. Empathy can be shown in both verbal and non-verbal ways. Three key points of empathy are having a client know that they are seen, heard, and accepted.

Being seen means not only physically seen, but seen as an individual person and not just a "client". Ways to show back to the client that they are seen are to comment on a unique aspect of that individual *unrelated* to their pet's medical concerns, such as their clothing (for example, if they are wearing a sport team's logo), their pet's collar, etc. It is also important that the client knows they are seen as a person when it comes to the medical issues, and that their emotions about these issues are noticed. A good way to show this by verbally stating what is seen: "you seem worried", "you look very scared". While these statements seem awkward or obvious at first, this technique is greatly appreciated by clients. Firstly, clients will appreciate the empathy and bond more with the clinician that shows it. Secondly, by bringing the emotion to the forefront, it can be dealt with and thus allow the client to feel it more and move on in the discussion about their pet. A statement as simple as 'I can see this is difficult for you; it can be very hard to hear that someone so close to you has cancer,' can be grounding for a client and may allow them to refocus on the medical conversation. Other non-verbal means to show a client they are seen is to use appropriate speaking tone and rate (e.g. quiet slower speaking in times of sadness) or posture in response to the client. Handing a box of tissues to a crying client is a great means of showing empathy.

Being heard is a similar concept to being seen; the veterinarian not only needs to listen to the client, but needs to show them that they are heard, and ideally understood. This process is closely tied to one of the other core skills, reflective listening (see below). Listen and take in what the client is saying and then show appreciation and understanding of their experience. This showing to the client can be accomplished both verbally and non-verbally. Reflective listening (below) describes the verbal showing. Non-verbal means include appropriate facial expressions, nodding, leaning in towards the client, etc.

Being accepted is an inherent desire in people, and this acceptance will help an owner to be open and honest with the clinician and help them also to voice concerns or questions that they may be embarrassed to mention otherwise. It also helps clients to work through difficult problems and see the veterinarian as supportive as opposed to adversarial. There are several categories of statements that one can make to help a client feel accepted, and intentionally using these statements will take conscious effort but can be of great benefit in improving the client-clinician bond. Three types of statements include 1.non-judgmental comment: "you were in a very difficult situation" 2. normalizing comment: "it is so common for people to miss these masses until they get very large" and 3.self-disclosing comment: "my cat has behavioral issues too".

These techniques for expressing empathy become even more critical with clients for whom it is difficult to feel empathy; verbally showing acceptance via the above phrases can help the client-veterinarian bond even for clients with whom it is very hard to bond. Note that showing a client that they are seen, heard, and accepted is also tied to reflective listening (see below).

Open vs Closed Ended Questions

Open ended questions are very important in obtaining an accurate history, as well as during discussions regarding diagnostics and treatment choices. Additionally, they greatly increase the client-veterinarian bond by allowing a client to tell their story – a key part to feeling understood and valued as a person. This "story telling" does not need to be very long or unguided; with specific techniques a veterinarian can guide the answers in situations where they may be too long. Very often, open ended questions SAVE time in an interview, as they allow the veterinarian to understand the true nature of the client's concerns without misleading results that the use of only closed-ended questions may provide.

Closed ended questions begin with words such as "when, is, did, who, where". These questions result in very short answers, often times "yes" or "no" or other one word answers. Closed-ended questions create an atmosphere that is more like an interrogation than an interview, and they are very much centered on what the *clinician* wants to know. These questions certainly play a role in an interview – they are helpful in emergency-type situations as well as in clarifying information. They are best used AFTER open-ended questions.

Open ended questions often begin with "what or how". Avoiding "why" is a good idea, as that word seems to carry judgment ("why did you do that?"). Open ended questions allow the client to talk and express their thoughts and concerns. While the client is "telling their story" they will use their own natural vocabulary; this allows the clinician to ascertain what type of vocabulary to use in return when speaking to the client for greatest understanding. A very helpful way to start a visit with a client is to ask a big, broad question such as "What has been going on?". If you already know the complaint (e.g., technician took a brief history; the patient is hematuric), an open ended question can still be asked while including the information you have: "So, Fluffy is having blood in her urine. Can you tell me what has been going on?". This technique both allows the client to know that you know their concern, and also allows them to share more and feel that their thoughts are worthwhile. Open ended questions are a powerful tool in improving a bond with the client, versus a rapid fire, just-the-facts interrogation style: "When did you notice the blood? Is she drinking more? Any straining?" etc.

Open-ended questions continue to be very helpful after the initial interview. Even with ongoing cases and familiar owners, don't assume an understanding of what the client is thinking. Open ended questions can avoid misunderstanding in difficult discussions. For example, rather than "Are you thinking about euthanasia?" and having an owner get hysterical because they were not, ask "what are your thoughts about options from here?". Also, "ask rather than tell" is a great rule of thumb. When a new diagnosis has been made, asking a client what they know about the disease, rather than jumping into a description of the problem for the client, can save time in a discussion and avoid unnecessary client education. This asking not only allows the clinician to begin the discussion at an appropriate level, but also importantly shows the client that their knowledge is valued – this leads to a better client/veterinarian bond as the client feels treated as a unique and important individual.

Reflective Listening

Reflective listening is the technique, also called active listening, by which an individual "reflects" back what has been said, thus showing interest in and *understanding of* the meaning of what the speaker is saying. With this technique, the listener repeats back to the client what was said or even implied, often via paraphrasing. This technique is a key component of showing empathy, as stated previously. Also, reflective listening is an excellent tool for checking understanding by the veterinarian. Reflective statements not only allow the client to know they are heard and seen, to "see" how they seem to the veterinarian, but they also allow the client to correct any misconceptions and add to the veterinarian's summary if needed.

A few simple tricks of reflective listening include non-verbals stated above, such as nods, smiles, mm-hmm's, and hand gestures. Also, when unsure what to say, or wanting an owner to expound without your input, a simple verbal repeat of the last word of phrase said can be helpful: Client : "Momo has just been acting crazy." Clinician: "Crazy?"

Reflective statements can seem awkward at first; having a "stem" with which to begin is very helpful. The classic stem is "what I hear you saying is…" Other stems may feel more natural (less like a therapist!) and include "so, you are saying…" "it sounds like…" "you are...". A complete statement could be something like: "It sounds like you are worried that Turner is having a poor quality of life right now." The client can then correct if that is not their main concern, or can feel a sense of relief that the clinician understands them if that is the concern. Another example of a reflective statement in action is: "So you were concerned about the cost of the treatment?" This may elicit a "Yes, it seems very expensive" or maybe "No, the cost isn't the problem, it is the time involved". Thus, the true concern can be addressed. This paraphrasing is one of the most effective listening tools available when one wants to convey to someone that what they are saying is important, and it is also an excellent way to verify correct understanding of what the client meant to express, which leads to improved diagnostic and therapeutic patient management.

LYMPHOMA

In dogs, the multicentric form of lymphoma (multiple peripheral lymph nodes (LNs)) is the most common. Typically, dogs are asymptomatic and present because their owners have noticed enlarged LNs ("lumps under chin"), or the LNs are noted to be enlarged on a routine physical exam. Less commonly, dogs with multicentric LSA may present clinically ill (decreased appetite, lethargy). Cytology is usually diagnostic, and immunocytochemistry can be used in the diagnosis as well as determining immunophenotype, which correlates with prognosis. Multidrug chemotherapy provides a median survival time of 12 months, with 25% of dogs living 2 years.

T cell vs B cell: One study reported on response in 41 dogs to single-agent doxorubicin in T vs B cell LSA. 25/29 (86.2%) of B cell LSA went into CR, while only 2/12 (17%) of T cell LSA had a CR. Overall response rate (complete + partial responses) was 100% for B cell vs 50% for T cell. Thus, doxorubicin may not be a very effective part of a multi-drug protocol for T cell LSA, and close monitoring for response to therapy is indicated.

Maintenance therapy: In the past 5-10 years, a shift has occurred away from protocols with an extended maintenance phase in which treatments are given at progressively longer intervals to protocols that are more intensified early on and last for a shortened duration (e.g. 4-6 months vs years). Recent studies have shown that shorter protocols result in similar disease free and survival times when compared to protocols with prolonged maintenance. The author's preferred protocol (modified UW Madison) entails 8 weekly treatments, then 8 treatments every other week for a total treatment duration of 6 months.

Rescue therapy: Rescue treatment is given when an animal comes out of remission or fails to respond to the initial chemotherapy protocol chosen. Depending upon the length of time the animal was in remission and if they have been able to be off of drugs, the original induction protocol or other drugs will be recommended. Anywhere from 50-100% of animals (depending on the protocol) experience a 2nd complete remission, which usually will be shorter than the first. A recent retrospective study of 95 dogs retreated with a CHOP-based protocol showed a 78% CR rate, with a median response duration of 159 days. The duration of time off of chemotherapy (after completion of the first CHOP round) was associated with the likelihood of a second response, with a median time off of 140 days for dogs that achieved CR vs. 84 days off for non-responders. Dogs that had a longer first remission duration (≥ 289 days) had a longer second remission duration (median 214 days vs 98 days for dogs with <289 days initial remission). Whether to try to obtain a 2nd, 3rd, 4th, etc remission is a decision each owner needs to make, once presented with the costs/side effects/likely response to the protocol.

New treatments on the horizon: Dogs with LSA respond well to chemotherapy and have a good quality of life throughout most of their treatments; however, a wall has been hit in regards to improving survival time by tweaking chemotherapy protocols. Bone marrow transplantation in combination with high dose chemotherapy for pet dogs with LSA has recently been used at NC State University; some individual dogs do very well with this therapy, but overall the responses are disappointing. Rabacfosadine (Tanovea™), a novel nucleoside analogue, showed an 81% overall response rate with a 200 day progression free

interval when alternated with doxorubicin for naïve lymphoma. This drug is likely to become FDA approved within the next year.

HEMANGIOSARCOMA

About 50% of HSA are splenic, about 25% are right atrial, and 25% are cutaneous.

Differentials for Splenic Mass: As a general rule, 1/3 of splenic masses in dogs are benign (hematomas), 2/3 are malignant. 2/3 of the malignant ones are HSA, with the other 1/3 comprised of an assortment of other tumors including but not restricted to leiomyosarcoma, lymphoma, plasma cell tumor, and histiocytic tumors. The odds of malignancy increased to ¾ in one study if the mass had ruptured and the patient was anemic. A more recent study of 71 dogs with a splenic mass and hemoabdomen needing a blood transfusion confirmed the findings of ¾ of the masses being malignant, with ¼ being benign. 93% of the dogs with malignancy had HSA; these dogs had significantly lower total solids and platelet concentrations than dogs with benign disease. Additionally, hemoabdomen can also be caused by a number of diseases; a recent review of the final diagnosis in 60 dogs with hemoabdomen showed 63% HSA (splenic and/or hepatic, less frequently omentum or kidney), 27% splenic hematoma, 5% splenic torsion, 3 % hepatocellular carcinoma, and 1.6% carcinomatosis. Thus, in this patient population, benign disease accounted for 33% of the cases of hemoabdomen; a finding generally considered very suspicious for HSA.

Differentials for Pericardial Effusion: Most (60 to 70%) clinically significant pericardial effusions are secondary to cancers, including right atrial hemangiosarcoma, heart base tumors (aortic body tumors, or chemodectomas), and mesothelioma. Idiopathic (benign) pericardial effusions make up about 30% of cases. Hemangiosarcoma is the most common cardiac tumor (10x greater than next tumor). Cardiac troponin I (cTnI) in the serum is a sensitive and specific marker for myocardial ischemia and necrosis and was found to be significantly elevated in dogs with pericardial effusion due to HSA (2.77 ng/dL; range: 0.09–47.118 ng/dL) compared to dogs with idiopathic effusion(0.05 ng/dL; range: 0.03–0.09 ng/dL). This serum test may be useful in differentiating dogs with HSA vs idiopathic effusion in cases where a mass cannot be detected.

Abdomino/pericardiocentesis: Fluid that is removed can be evaluated via a hematocrit tube for PCV and total protein; if the fluid is hemorrhagic the sample should be watched to see if clotting occurs. If it does, it is a fresh blood sample and not from free abdominal fluid; another sample should be obtained. Hemorrhagic fluid (PCV > 15 or so) very rarely reveals anything noteworthy on cytology. In critical cases, looking at a slide of the fluid under a microscope is usually not worth the time. If the sample is not hemorrhagic, cytologic analysis can be very helpful.

Imaging for these tumors: Ultrasound is a highly sensitive, noninvasive technique to image splenic and hepatic lesions. With HSA, these masses usually have a mixed pattern with anechoic to hyperechoic areas. “Target lesions” in the liver are most consistent with metastatic neoplasia. However, there are many times when lesions in the liver have an appearance that may be benign or malignant. Contrast harmonic imaging (or “contrast ultrasound”) involves administration of an IV ultrasound contrast medium (such as Definity or Sonovue) with subsequent imaging with contrast harmonic software on a conventional ultrasound machine. Contrast ultrasound may not only be able to differentiate benign from malignant liver nodules after detection, with close to 100% specificity, but may lead to improvement in the detectability of such metastatic nodules. Unfortunately, contrast ultrasound has not been shown to be helpful with splenic masses (benign vs malignant). Echocardiography is the best way to evaluate for right atrial lesions but still can provide a false negative unless pericardial effusion is surrounding the mass, making it easier to image. In one study, just 4/83 (5%) dogs with splenic HSA had echo be the only test that identified metastasis or a second primary tumor in the right atrium.

Diagnosis: Fine needle aspiration with cytology of HSA is typically very low yield. Masses in the liver or spleen that have a more solid appearance are more likely to provide cells and a diagnosis with cytology. Tumors that can be diagnosed in this manner include lymphoma, plasma cell tumors, and osteosarcoma. The definitive diagnosis of HSA requires exploratory surgery with an excisional biopsy. Histopathology can be challenging as HSA will bleed, causing hematomas around the tumor. The ENTIRE mass must be evaluated before a diagnosis of a benign hematoma vs. HSA can be believed.

Treatment and Prognosis:

Surgery

Splenectomy alone results in MSTs of only 19 to 86 days, with only ~6% of dogs living to one year. Rupture of the tumor did not correlate with a worse prognosis in cases treated with surgery alone in several studies. For right auricular masses, the mass may be able to be excised, with a MST of 4 months. Pericardiectomy alone can help relieve the signs of tamponade, and provides a similar MST.

Chemotherapy

Chemotherapy post-operatively for HSA (doxorubicin/cyclophosphamide) can extend the MST to 5-8 months. In a retrospective study, epirubicin (30 mg/m2) every 3 weeks for up to 4 to 6 treatments was used in 18 dogs, while 41 dogs received splenectomy alone. The MST of dogs given epirubicin (144 days) was greater than the splenectomy alone dogs (86 days). For all dogs, MST with stage I disease (345 days) was significantly longer than for dogs with either stage II (93 days) or III disease (68 days). Dogs with stage I disease treated with splenectomy alone had a MST of 238 days, greater than the 86 days previously reported, and potentially signifying an improved survival for this stage of splenic HSA. Impressively, the MST for dogs with stage I HSA treated with epirubicin was 983 days, and 4 of 7 of these dogs survived > 1 year. Seven of 18 dogs were hospitalized for epirubicin-induced GI toxicity.

Low dose continuous chemotherapy, or metronomic chemotherapy, may be as effective in dogs with post-operative HSA compared to doxorubicin. Etoposide (50 mg/m2 PO daily for 3-weeks) alternated with cyclophosphamide (12.5 to 25 mg/m2 per day orally for 3 weeks). Piroxicam (0.3 mg/kg PO once daily) was administered throughout. Both the MST and the median DFI in 9 dogs treated with the metronomic therapy were 178 days. By comparison, the MST and DFI in 24 historical control dogs treated with doxorubicin were 133 and 126 days, respectively.

MAST CELL TUMORS

MCTs are the most common malignant skin cancer in dogs, and significant variability exists in their biological behavior. Most of the tumors are cured with appropriate local therapy, but a subset shows very malignant behavior and the potential to spread to lymph nodes, liver, spleen, bone marrow, and other areas and to thus become a systemic cancer. Some factors that are considered to carry a more guarded prognosis in dogs with MCTs include recent, rapid tumor growth; certain locations (mucocutaneous, muzzle, pinnae, inguinal, nail bed); fixed ulcerated tumors; and lymph node involvement. A histologic grading scheme (grades I to III) was developed for classification of MCTs affecting haired skin and is still one of the strongest prognostic indicators of canine MCT behavior. However, histologic assessment is prone to operator subjectivity, giving rise to variable grading results for the same tumor among different pathologists. This subjectivity and variability between pathologists bring into question the heavy reliance placed on the grade of an MCT to predict its behavior and thus the need for additional therapies beyond primary tumor excision. In addition, most MCTs are designated grade II and most of these are cured with surgery. However, a subset of grade II tumors are aggressive like grade III tumors. Thus, many other prognostic factors, including various proliferative indices, DNA aneuploidy, c-kit–staining pattern, microvessel density, and mitotic index (MI), have been evaluated in an attempt to better predict the behavior of canine MCTs and to pick out the “bad” grade II tumors. The two-tier (low vs high) grading system has shown value in predicting behavior, and may be helpful in combination with the 3-grade system. Two groups evaluated just MI (total number of mitotic figures found in 10 high-power fields) and found that it is predictive of survival time, even within the grade II tumor category. The groups found slightly different values for the index, with the first paper showing a median survival of > 70 months for an index score <5 and a survival < 2 months for a score ≥ 5. The second group wrote a letter to the editor in response to the first paper and confirmed that high scores have very short survival times, with their index scores broken down into three groups: MI=0 = median survival time not reached (i.e. most of the dogs did not die of their disease) MI=1 to 7, median survival time of 18 months; and MI > 7, median survival of 3 months.

Additional therapy (chemotherapy, tyrosine kinase inhibitor (TKI) therapy) needs to be considered in dogs with poor prognostic indicators (e.g., grade III, high MI, poor location) after complete excision of the MCT. Dogs with nonexcisable tumors may also be considered for chemotherapy or TKI therapy; both of these therapies show better success against smaller tumors, but certainly can be beneficial in cases with bulky tumors.