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Biopsy of cutaneous lesions is often the most direct method for establishing a diagnosis of primary dermatologic diseases, as well as systemic diseases with dermatologic manifestations. The skin is readily accessible and typically heals without defect. In many cases, a diagnostic biopsy can be obtained with local anesthesia alone (mild patient, biopsy from trunk), light sedation and local anesthesia (wild patient, biopsy from ventrum), or full anesthesia (biopsy from face, paws, perineum). In addition, skin biopsies do not require expensive equipment or a high degree of technical skill. So, why are biopsies often shunned as a last resort diagnostic test? In many cases, skin biopsy is not performed because past experience has deadened the practitioner to the value of the technique. How many times have you submitted a perfect biopsy, certain of a definitive diagnosis, only to be disappointed by the dreaded “chronic inflammatory dermatitis,” or other non-committal diagnosis? How many times has the pathologist not even commented on your top differential diagnosis? This guide is designed to decrease frustration and maximize diagnostic value of skin biopsies.

PATIENT SELECTION

First, when should you biopsy? A skin biopsy should be performed on the very first visit if the primary lesion is depigmentation, vesicle/bullae, ulceration, necrosis, plaque, or nodule (not diagnosed by fine-needle aspirate cytology). A skin biopsy should also be performed whenever the clinician suspects neoplasia, life-threatening diseases, or immune-mediated dermatoses with significant morbidity. Basically, if your differential diagnosis list includes a serious disease that can be ruled in or ruled out with histopathology and skin biopsy is the most direct and effective method for establishing the diagnosis, don’t delay. Clinicians should also obtain a biopsy if empirical therapy for the top differential would cause serious harm if the condition is actually another disease high on the list of differentials. For example, don’t start immune suppressive therapy for suspected autoimmune disease, when the patient may have dermatophytosis or demodicosis. Get a biopsy diagnosis first!

Perform a biopsy on the second visit for any inflammatory dermatoses that has not responded to appropriate therapy for the initial diagnosis. In other words, if the condition did not resolve, then biopsy to find out why not! Along the same lines, you might consider performing a biopsy if you are the second or third veterinarian consulted by an owner for an unresolved dermatologic problem.

When not to biopsy? Biopsy should be delayed if there is a significant secondary bacterial infection. Bacteria typically bring large numbers of neutrophils and macrophages, potentially obliterating the histopathologic pattern caused by the primary disease. Your pathologist will not be able to differentiate primary lesion from response to infection. Therefore, if your cytology demonstrates significant bacterial overgrowth or infection, then delay biopsy until appropriate antibiotic therapy has resolved, or at least impacted, the bacteria component. Schedule for biopsy in 7-10 days; any remaining lesions are likely primary lesions that will therefore have a greater chance to yield a diagnostic specimen.

Also, delay biopsy if the patient is receiving corticosteroids. Corticosteroids suppress inflammation in primary lesions and may decrease the diagnostic value of the histopathology. Therefore, if steroids are indicated, biopsy samples should be obtained prior to starting therapy. If the patient is already on steroids, then discontinue therapy as appropriate and schedule the biopsy. Instruct the owner to return immediately if “new” lesions develop during the withdrawal period, as these are likely primary lesions.

SITE SELECTION AND TECHNIQUE

Once you have decided to biopsy there are several common errors to avoid and some useful techniques to improve the diagnostic value of samples collected. Different diseases have characteristic histopathologic patterns. Your goal is to present a sample with that pattern intact for visualization by the pathologist. Keep in mind these patterns may change with progression of disease, or be altered by secondary changes, such as exoriation, infection, ulceration, or artifacts created by sampling technique and fixation. In order to maximize the opportunity for the pathologist to see the diagnostic pattern, several simple rules must be followed: (1) don’t scrub or prepare the area, (2) use a fresh sharp biopsy punch, (3) be gentle, (4) put what you want the pathologist to see in the center of the punch, (5) select earliest primary lesion possible then additional samples of various ages, (6) submit multiple samples, and (7) place the samples immediately in formalin.

Sterile preparation of the biopsy site is a common mistake that should be avoided. Skin biopsies are clean procedure, but are not a sterile procedure. Clipping and scrubbing the site, removes scale and crust, rupture pustules, and can otherwise disrupt histopathologic features necessary for a diagnosis. If you want to be sure you never diagnose pemphigus or hepatocutaneous syndrome, keep scrubbing your biopsy sites. Caution should be taken if the patient is severely immune compromised, predisposed to septicemia, vegetative valvular endocarditis, or has orthopedic implants.

Using a new biopsy punch for each patient is strongly recommended. A punch is basically a round surgical blade; multiple use and resterilization dulls this blade just like it would a disposable scalpel blade. Dull punches, tend to gnaw through tough epidermis and dermis, causing mechanical damage and artifacts that decrease the diagnostic value of the sample. A sharp punch makes a cleaner cut, preserving architecture. However, the same punch can be used multiple times on the same patient before it gets dull. If neoplasia is suspected, a new punch must be used for each site. Recycling dull punches is false economy.

After making a clean cut through the epidermis and dermis, be careful not to crush the tissue by grasping across the middle of the specimen with forceps. Ideally extract the specimen with fine instruments, such as ophthalmology forceps and iris scissors. Insert the forceps between the specimen and the skin and attempt to grasp the tissue at the level of the subcutis or at the very edge of the dermis. Gently lift the specimen up and use the iris scissors to severe any cause on the lesion. If the specimen is large, you may use forceps to lift the specimen up and use the iris scissors to sever any cause on the lesion. Once you have a good sized specimen, you can use forceps to hold the specimen and transect it through the subcutis.

When taking a biopsy, be certain to place what you want the pathologist to evaluate directly in the center of the punch. A punch provides a round specimen. Laboratory personnel usually take the round specimen and transect it through the
center, leaving two, roughly symmetrical, semi-circular pieces. The flat side of each piece is placed down in the cassette, which eventually is the surface the pathologist will examine. Therefore it is critical to center the punch over the primary lesion (pustule, papule, etc). Frequently practitioners try to bridge normal and abnormal tissue in an attempt to capture the transition zone of disease. Unfortunately, what looks like obvious transition between normal and abnormal skin at the time the biopsy was obtained, looks grey and undifferentiated after 24 hours in formalin. If bridging normal and abnormal tissue seems vitally important, then collect a wedge biopsy with the long axis of the wedge perpendicular to the line of transition. Otherwise, when using a round punch select only the abnormal tissue.

Site selection is critically important to making a diagnosis, since selecting the most representative specimen gives the pathologist the best chance of seeing the diagnostic pattern. Choose primary lesions, such as depigmented skin, erythema, papules, pustules, vesicles, plaques, and nodules. Avoid secondary lesions, such as erosions, ulcerations, and excoriations. For example, in diseases characterized by nasal depigmentation and mucocutaneous ulceration, the slate grey tissue on the nasal planum is the perfect choice, followed by erythematous areas adjacent to ulcers. The ulcers themselves are the least valuable specimen, as all ulcers look the same to a pathologist. They see denuded dermis with serocellular crust, and diffuse inflammation. In general, newly formed lesions are the best samples, as they have had less time to develop confusing secondary changes and likely will demonstrate the primary disease process. It is prudent, however, to collect a few older lesions as they may also show important diagnostic features that characteristically develop later in disease progression.

Another common mistake made by practitioners is failure to submit enough samples. Even if all lesions on the dog look the same on gross examination, each one may vary significantly in histopathologic pattern. A 4 or 6 mm specimen is relatively small snapshot of the skin. There is the possibility that any random single sample may not provide a diagnostic pattern, even when taken from the most classic looking lesion. The best way to overcome this problem is to collect several punches from different areas, reducing the odds of a missed diagnosis, by giving the pathologist several views. Three punch biopsies should be considered the minimum acceptable number; five should be average.

The last point, placing samples immediately in formalin, seems obvious; however, a common mistake made when taking multiple samples is to place the specimens onto a gauze square as they are collected, then placing all of them into formalin when the procedure is complete. Desiccation and autolysis can alter diagnostic quality of specimens in less than five minutes; especially if the tissue is sitting under surgical lights. Improper fixation can also impact diagnostic quality of the specimen. With prolonged storage formalin breaks down to formic acid and paraformaldehyde, which provide less than adequate fixation of tissues. Formalin should not be kept for longer than one year before usage. Additionally, the tissue to formalin ratio should ideally be close to 1:20, with 1:10 being a minimum. If you practice in an area prone to freezing temperatures, 95% ethyl alcohol should be added to formalin prior to shipping fixed tissues. One part ethyl alcohol to nine parts formalin is sufficient.

SUBMISSION TO PATHOLOGIST

Always, always, always, give the pathologist a detailed history along with the specimens. Include description of lesions, distribution, region sampled, duration of clinical signs, other diagnostic findings (skin scraping, cytology, culture, etc), concurrent systemic illness, response to therapy, and most importantly your top differential diagnoses. If you want the pathologist to rule in or rule out a specific disease, you must tell them what you are thinking about. Some pathologists may even request special stains early on if they know you are concerned about a specific infectious disease or tumor type.

Finally, in order to maximize the diagnostic potential of your skin biopsies, send your samples to a pathologist with a special interest in dermatology. Dermatopathology is a subspecialty of pathology, and skill in interpretation of patterns of dermatologic diseases may vary from pathologist to pathologist depending on his or her level of interest in the diseases.