Better biopsies

Geoff Orbell

A common frustration for both clinicians and pathologists are non-diagnostic biopsies for dermatology cases and skin masses. This talk will discuss the most common causes from a pathologist's perspective, how to prevent them, which instruments or techniques are most appropriate given the clinical presentation and the importance of providing a relevant clinical history with any submission to a diagnostic laboratory and communicating with the pathologist if the results do not make sense clinically.

Causes of non-diagnostic biopsies

Lack of appropriate history or providing incorrect information

1. 88% in one veterinary study.
2. 80% in one medical study.
    a. 20% of diagnostic errors in medical biopsies were due to inadequate clinical history provision.
        i. Leads to litigation of the submitter for misdiagnoses.
            - Also happening with veterinary diagnostic laboratories.
    b. 32% of cases had significantly increased turnaround times for results.
        i. Running unnecessary additional tests.
        ii. Increased laboratory costs.
3. Laboratory submission forms are legal documents, and diagnostic labs have a requirement to hold them for a minimum of seven years.
4. Signalment
    a. Species
    b. Sex
    c. Age
    d. Breed
5. Clinical history.
    a. Clinical signs
    b. Duration
    c. Response to treatment
    d. Current or recent medications
    e. Ancillary diagnostics, e.g. blood tests, endocrine function.
6. List differential diagnoses.
    a. At the very least histology should be able to rule some out.
7. Send photos!
8. Location
    a. Organ affected, e.g. skin, liver, spleen.
    b. Anatomic site
        i. Dermal vs. subcutis, e.g. melanomas, MCTs.
9. Lesion(s) description.
    a. Primary vs. secondary.
        i. Papules, macules, masses, plaques, alopecia.
    b. Size
    c. Distribution
    d. Consistency, e.g. solid vs. cystic.

10. Primary vs. secondary lesions
    a. Primary
        i. Papules, macules, patches, nodules, plaques, pustules, vesicles, bullae, erosions.
    b. Secondary
        i. Ulcers, fistula, furuncles, scarring, collarettes, lichenification.
    c. Can be both.
        i. Alopecia, depigmentation, seborrhoea, comedones.
11. Well or poorly circumscribed lesions.

Lack of tissue preservation

1. Autolysis
    a. Inadequate formalin.
        i. Large specimens - Splenic masses, soft tissue sarcomas, mammary masses/strips.
            - Fix intact in-clinic in large bucket of formalin.
            - Drain and submit whole triple bagged ziplock bags.
2. Necrosis
3. Artefact
    a. Sampling
        i. 90% of artefacts.
            - Shearing (punch biopsies).
            - Crush (rat-tooth forceps).
            - Diathermy
                - Cooks the tissue.
                - Don’t use in excisional biopsies with margins <5mm as cannot assess accurately for complete excision.
            - Post-surgical incision.
                - Don’t cut into biopsies after surgery to speed up fixation or look for foreign bodies.
                  Tissue will distort during fixation due to collagen cross-linking.
                  Results in wide gaping of any post-surgical incision.
                  Means surgical margins cannot be accurately assessed.
    b. Processing
        i. Small samples can be lost through cassette
            - Use mesh cassettes
        ii. Orientation
            - Small biopsies cannot be trimmed therefore can rotate during processing
        iii. Mineralised/bony samples can chip out

Non-representative samples

1. Difficult site.
    a. Blind nasal biopsies.
        i. Inappropriate technique.
2. Incisional instead of excisional.
3. Inappropriate instrumentation.
4. Inappropriate biopsy size or number.
5. Inappropriate site.
    a. Biopsies of secondary lesions, e.g. scar tissue, furunculosis.
        i. Misidentification of tissue.
            - Biopsy salivary gland instead of mandibular lymph node.
    b. Mixed up cases.
        i. Always label biopsy container with patient’s name and check.

Genuinely non-diagnostic

1. Lipomas vs. adipose tissue.
    a. Incisional cannot differentiate histologically.
    b. Excisional margins cannot be differentiated.
        i. Ink margins.
2. Granulation tissue.
3. Fibrosis
4. Normal tissue.

Specific tissue biopsies

Skin biopsies for histology

1. Don't biopsy
    - Suspected hypersensitivities
        - Histology cannot reliably differentiate underlying cause
    - Ulcerated lesions or fistulas
    - Chronic lichenified hyperpigmented skin
2. Medications
    - Corticosteroids
        - Ideally biopsy after:
            - 2-3 weeks off topical or oral corticosteroids
            - 6-8 weeks after depot corticosteroid preparations
    - Antibiotics
        - Superficial pyodermas are almost always secondary to some underlying cause but histologically obscure primary lesions.
            - Identify pyoderma by cytology.
        - Pretreat with antibiotics to remove secondary infection prior to biopsying just before end of course.
3. Don't clip or surgically scrub.
    - Removes crusts and can cause artefact to epidermis.
    - Include crusts with histology biopsies even if they become dislodge during the biopsy process.
4. Biopsy intact primary lesions.
    - Primary lesions:
        - Papules, macules, patches, plaques, depigmentation.
        - Vesicles, pustules, bullae.
        - Folliculitis, hyperkeratosis, seborrhoea, comedones.
        - Nodules, tumours.
    - Secondary lesions:
        - Ulcers, fistulae, furunculosis.
        - Collarettes and crusts.
    - Secondary infection can mask the underlying primary process.
        - Misses primary epidermal changes.
        - Do cytology first and if superficial pyoderma present then pretreat with antibiotics and biopsy just before finishing the course.
5. Skin punches
    - Best for most primary dermatology lesions except.
        - Vesicles, bullae or pustules (unless very small).
        - Shearing force of biopsy punches can rupture these lesions.
        - Tumours
            - Shearing artefact.
    - Use 8mm Skin punch biopsies wherever possible.
    - Use a new biopsy punch for each patient.
    - Lab technician needs to be able to bisect biopsy parallel with the direction of hair growth so pathologist can visualise the entire length of hair follicles histologically.
    - Direction of hair growth difficult in the lab especially if alopecic.

- Draw a line on skin with narrow nibbed permanent marker (Sharpie) before biopsying to show direction of hair growth.

6. Incisional biopsies
   - Margins of ruptured vesicles, ulcerated/non-ulcerated areas if that is only available lesions.
   - Margins of depigmenting lesions.
   - Deep lesions where skin punch can't reach, e.g. deep pyoderma/panniculitis/vasculitis.
   - Reliant of accurate clinical identification of best biopsy sites/primary lesions.
   - Deep wedge biopsies great for non-haired skin lesions eg nasal planum/footpads.
     - Orientation not as important as haired skin.
     - Easier to close.
7. Excisional biopsies
   - Best chance of diagnosis and surgical cure.
   - Best for biopsying intact pustules/vesicles.
8. Biopsy the centre of the lesions especially in alopecia cases.
   - Also take a biopsy of normal.
9. Always take multiple biopsies and identify sites.
10. Drill biopsy punch in one direction only to minimise shearing artefact.
11. Ensure adequate formalin: tissue ratio.
    - Use 10% neutral buffered formalin.
    - Don't stick biopsies to cardboard or tongue depressor.
12. Don't grab biopsy with rat tooth forceps.
    - Elevate from underneath.

Skin biopsies for tissue culture

1. Surgically scrub to minimise skin commensal contamination.
2. Avoid ulcerated/fistulated lesions.
   - Biopsy adjacent to them.
3. Use new 8mm punches or incisional biopsies.
4. After biopsy is removed lay it on its side on a sterile surface and cut off epidermis with scalpel.
   - Minimises skin commensal contamination.
5. Place biopsy in the liquid media from an E-Swab or a sterile saline soaked surgical swab inside a sterile container.

Lymph nodes

1. Most commonly biopsies for suspect lymphoma or metastatic neoplasia.
2. Excisional biopsies only.
   - Architecture required for identification of indolent lymphomas.
   - Early metastatic neoplasia can be very focal and just in subcapsular sinuses so can be missed.
   - Punch and Tru-cut biopsies cause shearing and crush artefact in lymph nodes.

Gastrointestinal biopsies

Table 1. Advantages and disadvantages of endoscopic and incisional gastrointestinal biopsies.

Feature	Incisional	Endoscopic
Patient morbidity	More risky	Less risky
Biopsy site number	Less	More
Lesion ID for biopsies	Serosal, transmural, other organs	Mucosal only, sometimes too superficial, e.g. villi only
Biopsy site limitations	All GIT, other abdominal organs	Stomach, duodenum, colon, rectum
Tissue sampled	Full thickness	Mucosal only
Tissue artefacts	Rare	Common
Tissue processing	Easy to orientate	Impossible

5) Bone

- Use dedicated bone biopsy instruments.
  - Jamshidi needle 13G cats and smaller dogs to 8G in larger dogs.
- Biopsies for bone pathology.
  - Take multiple cores.
  - Sample areas of bone lysis not periosteal new bone formation.
- Bone marrow cytology and biopsies.
  - Remember to include EDTA for haematology and fresh blood smears.
  - Bone marrow cytology is extremely sensitive to formalin fumes.
    i. Keep formalin container in different room to surgery.
    ii. Take bone biopsy first but do not put in formalin straight away.
    iii. Take cytology samples from bone marrow (through hole created by biopsy) and roll biopsy on separate slides.
    iv. Get someone to prestain at least one slide to ensure there are intact nucleated cells present before finishing the surgery.
    v. Place prestained and unstained cytology slides and blood films in slideholder and place in ziplock specimen bag and keep separate to biopsy.
    vi. Place biopsy in formalin and send in different courier bag to cytology and blood smears.

Miscellaneous tips and tricks

1. Re-excisional biopsies of soft tissue sarcomas with incomplete margins.
   a. Often can't differentiate postoperative granulation tissue and fibrosis from residual tumour.
2. Iatrogenic trauma.
   a. Follicular cysts/keratising follicular tumours/fibroadexal hamartomas/calluses/acral lick granulomas have cystic hair follicles containing keratin.
      i. Easily excised with minimal margins when intact.
      ii. Trauma causes rupture of cystic hair follicles releasing free keratin into the dermis.
         - Can be iatrogenic with fine needle aspiration or owners/vets squeezing masses.
           - Keratin is extremely irritant and acts as microscopic foreign bodies that induces severe pyogranulomatous inflammation that extends along fascial planes.
           - Requires much more extensive surgical excision to excise.
         - Surgically excise early prior to trauma or soon after cytological diagnosis.

Inking biopsies

1. Identifies histological surgical margins for the pathologist.
   a. Dye sticks to but does not penetrate the tissue.
2. Different colours can be used for different margins or areas of interest for the surgeon.
3. Ideally ink the tissue before formalin fixation.
   a. Sticks better to fresh tissue.
   b. Margins will deform during the fixation process due to collagen cross-linking.
4. Commercial tissue dyes work best Eg Davidsons, Mark-It or Grale tissue dye.
5. Process
   a. Blot specimen dry after excision.
   b. Use tissue dye-soaked cotton-top swab or small paintbrush to mark the lateral and then deep surgical margins including skin margins.
   c. Blot excess ink from the sample.
   d. Allow to dry for 5–15 minutes.
   e. Place into formalin container with at least a 10:1 ratio of formalin: tissue.

References

Brannick *et al*. Influence of submission form characteristics on clinical information received in biopsy accession. *Journal of Veterinary Diagnostic Investigation* 24(6): 1073-1082, 2012
Craft WF *et a*l. Skin biopsy guidelines: tips and advice from veterinary pathologists to practitioners. *Journal of the American Veterinary Medical Association* 261(S1): S114–S121, 2023
Karcioglu ZA. A Problem That Is Older than the Hills: The Communication Gap between the Pathologist and the Surgeon. *Ocular Oncology and Pathology* 6(1): 1-2, 2020
Nakhleh RE *et al*. Necessity of clinical information in surgical pathology. *Archives of Pathology and Laboratory Medicine* 123(7): 615-619, 1999