GASTROINTESTINAL BIOPSY TECHNIQUES

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Gastrointestinal (GI) biopsies can provide information that cannot be gained from any other method. That being said, the need for biopsies has decreased through appropriate use of therapeutic trials. The need for full-thickness surgical biopsies has been further reduced through the use of endoscopy. When indicated, implementation of proper biopsy technique and sample preparation greatly enhances the quality and amount of information gained.

Generally, the decision to perform a biopsy is based on the presence of clinical signs attributable to the GI system that cannot be attributed to other body systems. These include chronic vomiting, weight loss, GI blood loss, gastroduodenal reflux or evidence of small intestinal diarrhoea. GI biopsy is probably the last step in making a diagnosis after other less-invasive tests have been exhausted unless there is evidence of obstruction, severe haemorrhage or perforation. The more clinically ill the patient is (i.e. severe weight loss, poor body condition score, hypoalbuminaemia, anorexia, ultrasonic evidence of infiltrative disease) the more likely therapeutic trials should be skipped and biopsies should be performed.

Patients of appropriate age which have clinical signs attributable to inflammatory bowel disease (IBD) should have therapeutic trials prior to biopsies. These include empirical treatment for Giardia and endoparasites, supplementation with cobalamin and folate, dietary trials, antibiotic trials, corticosteroids or other immunosuppressive medications.

When therapeutic trials have been unsuccessful, abdominal imaging (ultrasound or CT) should be performed to try to identify lesions which may not be accessible endoscopically, including lesions in the liver, spleen, lymph nodes, lower duodenum, jejunum, pancreas and/or kidneys. If clinical signs are attributable to the upper gastrointestinal tract, and abdominal imaging has eliminated lesions which may not be accessed, endoscopy with biopsies is generally preferred to full-thickness surgical biopsies. Endoscopy is contraindicated if there is evidence of GI perforation characterised by free abdominal air or peritonitis.

GASTROINTESTINAL BIOPSIES

General comments about gastrointestinal biopsies:
1. It is not always appropriate to biopsy every patient with chronic gastrointestinal disease
2. Biopsies are probably not appropriate for patients with upper gastrointestinal clinical signs of less than 3 weeks duration.
3. There can be great inter-observer variation in histologic findings between pathologists
4. Duodenal biopsies (compared with other GI sites) are often the most telling in dogs with chronic vomiting or small-bowel diarrhoea.
5. Biopsies of the ileum are generally recommended because they add significantly to the complete diagnosis of the patient even if lower intestinal signs are absent.
6. GI lymphoma is often localised in the ileocecal junction.
7. Ileal biopsies allow diagnosis of tumours and protein-losing enteropathy which may be missed on duodenal and colonic biopsies.
8. In most patients, duodenal and ileal biopsies are required, and must be of excellent quality.
9. Expectations from gastrointestinal biopsies
   A. Differentiate mycotic enteritis from lymphoma or IBD
   B. Differentiate moderate IBD from lymphoma
   C. Differentiate eosinophilic enteritis from lymphoma
   D. Immunophenotyping can help differentiate IBD from lymphoma
   E. Cannot expect to be able to accurately assess the severity of IBD or determine which patients will respond to therapy.

ENDOSCOPIC BIOPSIES

General comments about endoscopic biopsies
1. Smaller biopsy forceps (less than 2.5 mm) should not be used
2. Accuracy of endoscopic biopsies are highly operator dependent
3. Samples must include mucosa and submucosa
4. GI lymphoma appears to be primarily a submucosal disease; biopsies which only get the villous tips will likely miss the diagnosis.
5. The quality of endoscopic ileal biopsies are often superior to duodenal biopsies because the mucosa of the ileum is thinner, allowing more reliable inclusion of the submucosa
6. 7-8 samples from each location should be taken
7. Good biopsy samples should be pale and preferably white, long pieces of tissue, with solid rather than gelatinous consistency
8. As quality increases, the required number of samples decreases
9. The quality of endoscopic biopsies greatly influences the pathologist’s ability to diagnose gastrointestinal disease
10. It may be challenging in some areas of the GI tract to obtain samples which are deep enough because it can be difficult to orient the scope perpendicular to the mucosa
11. Some anatomical sites (duodenum, ileum) can be difficult to access by inexperienced clinicians
12. Submucosa can be difficult to access in dogs greater than 35 kg
13. Properly performed biopsies eliminate the need for surgical biopsies in 95% of cases where the endoscope can reach the lesion
14. If the duodenum or ileum cannot be entered, the biopsy forceps should be blindly advanced into the duodenum or ileum and multiple samples collected
15. One of the samples can be assessed cytologically using squash preparation for eosinophilic enteritis, adenocarcinoma, lymphoma, inflammatory cells or spirochetes.

**Submission of endoscopic samples**
A. When samples are collected, they should be handled carefully to minimise artefacts and distortion.
B. Tissue should be removed using a 25 gauge needle
C. Samples should be submitted in cassettes, not free-floating in formalin.
D. Tissue samples should be oriented luminal side away from the sponge in the cassette.
E. Cassettes are placed in 10% buffered formalin
F. Different locations are placed in different labelled vials
G. Small tissue samples should not be allowed to dry out
H. When endoscopic samples are submitted, the pathologist should report on the number and quality of samples to help the clinician to determine if technical modifications should be made to improve biopsy procedure

**Advantages of endoscopic biopsies include:**
1. Endoscopy is minimally invasive
2. Multiple biopsy samples can easily be collected
3. Morbidity and mortality from endoscopically collected gastrointestinal biopsies are very low with fewer complications than open surgical biopsies
4. Endoscopy allows visualisation of mucosal lesions prior to biopsy which can increase the diagnostic yield
5. Microbiological samples can be simultaneously collected
6. Ulcerations are easily visualised
7. Endoscopy allows photographic documentation of findings
8. Endoscopic biopsy allows detection of Helicobacter pylori
9. Endoscopy allows detection of gastric mucosal hypertrophy and duodenal polyps
10. Endoscopic appearance is better correlated with outcome in pets with IBD than histological diagnosis
11. Endoscopy procedures are generally shorter, less stressful and less expensive than surgical biopsy procedures

**Disadvantages of endoscopic biopsies include:**
1. Preparation of the gastrointestinal tract is absolutely required. Only fasting is required for the upper GI whereas fasting, enemas and ingestion of electrolyte solutions are required for colonoscopy
2. Functional disease cannot be diagnosed and luminal diameter cannot be determined
3. Generally not able to diagnose dietary responsive enteropathy, antibiotic responsive diarrhoea or GI motility disorders
4. Only mucosal and intraluminal diseases can be detected
5. Distal duodenal and jejunal lesions cannot be detected in most patients
6. Diffuse disease processes may be missed if only the stomach and duodenum are sampled
7. Biopsy size is limited
8. Not appropriate if perforation is suspected
9. Gastric distension may reduce venous blood return an induce vagal bradycardia
10. Can be easy to collect inadequate samples
OPEN SURGICAL FULL-THICKNESS BIOPSIES

Open biopsies should be reserved for the following circumstances:
1. Abdominal imaging suggests that lesions exist outside what is accessible via a scope
2. There is evidence of perforation
3. There is evidence of obstruction
4. The clinician lacks skill and expertise to enter and sample the duodenum and ileum
5. The results of endoscopy/biopsy inadequately explain the clinical picture
6. Cats with evidence of chronic gastrointestinal disease. One study in cats (Evans, 2006) showed that while endoscopic biopsies were adequate to diagnose gastric lymphoma, full-thickness biopsies were required to diagnose intestinal lymphoma. Lymphoma would have been missed in 9/11 cats if full-thickness intestinal biopsies had not been performed. It is not clear if the addition of ileal biopsies would have increased the diagnostic accuracy of endoscopic biopsies. Less than half of the cats with lymphoma had gastric lymphoma.

General considerations of surgical biopsies:
1. Stay sutures help immobilise the intestine for sample collection and intestinal suturing
2. Additional suture may be used in the proposed sample site to help elevate and retract it
3. The organ should be packed off prior to biopsy procedure to prevent spillage of intestinal contents
4. Omentalisation reduces postoperative leakage and should always be performed
5. Early postoperative feeding accelerates intestinal healing and reduces leakage and sepsis in humans
6. Consider alimentation during procedure
7. The entire abdomen should be evaluated visually from the cardia of the stomach to the terminal colon
8. Samples should be obtained from any grossly abnormal areas and from the stomach, duodenum, jejunum, ileum, mesenteric lymph nodes and liver (and pancreas in cats)
9. The stomach is generally closed in a two layer pattern
10. Intestine is generally closed in a single layer using a simple continuous pattern

Advantages of an open biopsy
1. A single biopsy in each of the following: stomach, duodenum, jejunum and ileum is usually sufficient
2. Open full-thickness biopsies are larger samples
3. The entire abdomen can be explored and biopsies performed
4. Risk is low (but not insignificant)
5. Surgical biopsies can be curative for focal disease
6. No specialised equipment is required
7. Ileal biopsies which are so important in diagnosing the cause of gastrointestinal signs can be easily acquired

Disadvantages of open full-thickness surgical biopsies
1. Cannot see mucosal lesions when deciding on location of biopsy
2. As many as 12% of patients have been reported to have intestinal dehiscence and leakage following full-thickness biopsy procedures (Shales CJ 2005). There were no risk factors identified.
3. Grossly abnormal areas can have disruption of the suture-holding submucosal layer which can make risk of biopsy higher
4. Inexperienced surgeons have significantly longer surgical times

Risk factors for leakage of intestinal biopsy procedures
1. Surgical technique
2. Hypoproteinaemia
3. Uraemia
4. Infection
5. Corticosteroid administration
6. Systemic disease
7. Malnutrition
8. Weight loss
9. Pre-existing peritonitis

COLONIC BIOPSIES
The primary indication for colonic endoscopy/biopsy is chronic large bowel disease unresponsive to appropriate dietary, antibacterial or anthelmintic therapies. Also includes patients that have haematochezia are losing weight or are hypoaluminaemic (possible protein losing enteropathy). Colonic endoscopy requires fairly involved preparation. Rigid biopsy forceps can be used to obtain excellent biopsy samples including submucosal lesions.
Endoscopy generally obviates the need for surgical biopsies as most of the required information can be acquired with rigid endoscope and flexible biopsies of the ileum. Flexible biopsy instruments can obtain mucosal samples but not submucosal samples of the colon. While the risk with surgical and endoscopic biopsies is relatively low, if the colon is severely ulcerated, biopsies should be taken with care because perforation may occur. There is a great deal of disparity between results of ileal and colonic biopsies. Therefore, ileal biopsies are required particularly in dogs with large-bowel diarrhoea and in cats with vomiting and diarrhoea. Generally, if the primary clinical signs are related to large-bowel disease, then endoscopic biopsies can be performed obviating the need for full-thickness surgical biopsies. If there is an indication for surgical abdominal exploration, then ileal and colonic full-thickness biopsies can be performed. In the author’s opinion, there is not a high risk with colonic biopsy as long as there are no pre-existing risk factors. There is, however, little advantage to full-thickness ileal and colonic biopsies over endoscopic biopsies if endoscopic biopsies can be reliably performed.

**LAPAROSCOPY**

Laparoscopy can be used to fully explore most of the abdomen. Biopsies from all abdominal organs can be performed. Gastrointestinal biopsies are performed by making a small abdominal incision and exteriorising the gastrointestinal organ to be sampled. Laparoscopy is generally limited by the availability of equipment and expertise. When preparing for laparoscopic surgery, the entire abdomen should be clipped and prepared in the event that the procedure needs to be converted to an open laparotomy.