The "GI Panel": Use, Abuse, and Interpretation

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The components of the "GI panel"

Most clinicians, when referring to a GI panel, are referring to the measurement of serum concentrations of specific pancreatic lipase immunoreactivity (Spec-cPL in the dog, Spec-fPL in the cat), serum trypsin-like immunoreactivity (cTLI in the dog, fTLI in the cat), and the serum concentrations of two water soluble, B-group vitamins, cobalamin (Vitamin B_{12}) and folate (Vitamin B_9). Together, these compounds can provide valuable information regarding the presence and localization of disease in the pancreas and small intestine, and they may also suggest the need for therapeutic supplementation. The normal physiology and significance of abnormalities of these compounds are discussed individually below. While most clinicians will use the full panel of all four compounds, particularly in cases where clinical signs are vague or inconsistent, in some situations it can be cost effective to measure only one of the pancreas markers. For instance, if the clinical suspicion is of exocrine pancreatic insufficiency in a dog, due to the presence of compatible clinical signs, little additional value is obtained from measuring Spec-cPL, cTLI is the test of choice. Equally, in a dog with a strong suspicion of pancreatitis there is usually little additional value in measuring cTLI and Spec-cPL is the test of choice. In the cat, however, the clinical signs and histories of both exocrine pancreatic insufficiency and pancreatitis are sufficiently vague and non-specific that it is generally advisable to at least initially measure both fTLI and Spec-fPL in this species.

Trypsin-like immunoreactivity (cTLI, fTLI)

The serum concentration of trypsin-like immunoreactivity represents the presence of (mainly) trypsinogen and (rarely) active trypsin in the circulation. Trypsinogen, the zymogen precursor to active trypsin, is essentially exclusively synthesized in the pancreatic acinar cells, where it is packaged in secretory granules before excretion in to the pancreatic duct system. Pancreatic acinar cellular damage, for instance with pancreatitis, can result in the loss of trypsinogen into the pancreatic interstitium and circulation, resulting in a higher than normal concentration. Loss of acinar cell mass, as occurs in both pancreatic acinar atrophy in dogs and as an end stage of chronic pancreatitis in dogs and cats, can result in subnormal concentrations of TLI. Detection of a serum TLI concentration <2.5µg/L is highly sensitive and specific for the diagnosis of exocrine pancreatic insufficiency in the dog. In the cat, a serum fTLI concentration ≤8 µg/L is suggestive of exocrine insufficiency. Values within the reference range, even if "low normal", rule out exocrine insufficiency due to reduced functional acinar cell mass.

Elevations in serum TLI will be seen in some animals with acute pancreatitis. Serum TLI concentrations rise rapidly early in the course of acute inflammatory disease of the pancreas, but also return to baseline relatively rapidly, and are typically at or slightly below baseline values within 48 to 72 hours after the onset of a bout. Consequently, a normal serum TLI concentration does not reliably rule out the presence of inflammatory pancreatic disease.[1] In the context of the GI panel, the greatest utility of the serum TLI concentration lies in the diagnosis or ruling out of exocrine pancreatic insufficiency as a cause of small intestinal diarrhea.

In some cats the serum fTLI concentration is mildly to moderately elevated, even though the clinical signs reported (diarrhea, weight loss) are more consistent with small intestinal disease. In many of these cats, serum Spec-fPL concentrations are normal. While the mechanism underlying this pattern of results is uncertain, it likely relates to a loss of normal negative feedback from the small intestine to the pancreas. This particular pattern of results (high TLI, normal PLI) in the cat is strongly suspicious of small intestinal disease, and warrants assessment of the serum cobalamin and folate concentrations.

Specific pancreatic lipase (Spec-cPL™, Spec-fPL™)

As with trypsin/trypsinogen, specific pancreatic lipase is synthesized only in the exocrine pancreas. Release of enzymes into the circulation is via leakage, and increased release is generally held to be consistent with acinar cellular damage occurring during pancreatitis. Generally speaking, serum concentrations of PLI show greater magnitudes of increase and longer durations of elevation above baseline than TLI in the same patient.

Detection of elevated serum concentrations of specific pancreatic lipase (fPLI or Spec-fPL) has a higher reported sensitivity and specificity than fTLI for diagnosis of pancreatitis in the cat. In one study, where fTLI achieved overall sensitivity and specificity of 28% and 82%, respectively, fPLI achieved overall sensitivity and specificity of 67% and 67%, respectively.[2] In the same study, sensitivity of fPLI for the diagnosis of "moderate to severe" pancreatitis was 100%. A larger study (n=182 cats) of the Spec fPL assay reported an overall sensitivity for this test of 79%, with a specificity of 82% for detection of pancreatitis in this group.[3] Overall, the Spec-fPL assay has the highest currently reported sensitivity and specificity of any diagnostic modality for the detection of pancreatitis in the cat.[1]

While pancreatic lipases are highly specific for the exocrine pancreas, the normal range of these assays in both dogs and cats includes values close to or equal to zero. Consequently, the Spec-c/fPL assays cannot be used to diagnose exocrine pancreatic

insufficiency. The main utility of the pancreatic lipase concentrations lies in the detection of exocrine pancreatic inflammation in both species, this is particularly valuable in the cat as clinical signs of pancreatitis in this species are often subtle or vague.

Serum folate

Folate is a water-soluble, B-group vitamin (Vitamin B_9) that is abundant in most small animal diets. As dietary deficiency of this vitamin is highly unlikely, the serum concentration of folate is an indicator of the small intestinal absorptive capacity for this vitamin. Folate monohydrate, the major form of folate absorbed from the small intestine, is absorbed exclusively via a receptor mediated process in the duodenum, thus a low serum folate concentration suggests a lack of duodenal receptors, and implies duodenal mucosal disease with a very high specificity.

Folate availability from the GI tract can be increased in some disease states. Many intestinal bacteria, including some *Lactobacillus* spp and representative flora from the large intestine, are net synthesizers of folate and release significant quantities of folate into their environment. In the dog an increased serum concentration of folate has traditionally been considered suggestive of bacterial overgrowth (see below), based on the assumption that a more "large intestinal" flora has migrated up into the small intestine. However, as mentioned above, some *Lactobacillus* organisms are net folate synthesizers as well as being "desirable" flora. With increasing use of partially fermentable fiber sources such as fructose-oligosaccharides in pet diets, there has been a population wide increase in serum folate concentrations.

Relatively recent studies of dogs with chronic enteropathy and suspected small intestinal bacterial overgrowth have found no difference is serum folate concentrations between dogs that responded to antibiotic therapy and those that did not.[4] In the author's experience at least, elevated folate concentrations are common in many animals with minimal to no evidence of typical "bacterial overgrowth", and this finding is of little impact to the management of clinical cases. The obverse of this observation, though, is that a low serum folate is highly meaningful, and a strong indicator of significant small intestinal disease of some form.

Serum cobalamin

Cobalamin is also a water-soluble, B-group vitamin (Vitamin B_{12}). In common with folate, this vitamin is abundant in small animal diets and it is extremely difficult to induce cobalamin deficiency in companion animals via dietary means. Also in common with folate, the serum concentration of cobalamin reflects the small intestinal absorptive capacity for this vitamin. Cobalamin undergoes a complex receptor-mediated absorptive process that occurs exclusively in the ileum in all species studied to date, including both dogs and cats. As the absorption of cobalamin occurs exclusively in the ileum, a low serum concentration of this vitamin strongly suggests ileal mucosal dysfunction.

Absorption of cobalamin relies on the formation of complexes between cobalamin and a binding protein called intrinsic factor, this protein is synthesized in the pancreas and gastric mucosa in dogs, [5] and exclusively in the pancreas in the cat. Thus exocrine pancreatic insufficiency is almost invariably associated with low cobalamin concentrations in cats.[6,7] As the clinical signs of exocrine insufficiency in many cats are vague and often dominated by weight loss and poor appetite, it is important to measure serum fTLI in cats with low cobalamin to help rule in/rule out this disease. While exocrine insufficiency is certainly a potential cause of low cobalamin in cats, it is not the primary cause. Infiltrative disease of the ileum, either inflammatory enteropathies or lymphoma, remain the most common cause of low serum cobalamin in cats and dogs.[8,9]

Some enteric bacteria, particularly some species of *Clostridium*, are able to degrade the cobalamin/intrinsic factor complexes and then utilize the cobalamin for their own needs, thus patients with the conditions referred to as "bacterial overgrowth" may present with low serum cobalamin due to bacterial competition. Decreased serum cobalamin concentration was identified in 16/29 dogs with chronic enteropathies, however there was no differences noted in dogs with differing definitive diagnoses.[4]

Cobalamin malabsorption can lead to a state of body-wide cobalamin deficiency, with deleterious effects on many cell types in the body, including enterocytes. Recognition of low serum cobalamin and parenteral supplementation to address this is an important part of the management of dogs with chronic enteropathies. Interestingly, low serum cobalamin concentration has been identified as a negative prognostic factor for dogs with chronic enteropathies,[10] and cats with gastrointestinal lymphoma. [11]

The combination of low serum cobalamin and folate concentrations is a very specific indicator of diffuse small intestinal mucosal pathology of some form. Any infiltrative disease, including the various forms of inflammatory bowel disease and intestinal lymphoma, may lead to this combination of abnormalities. Documentation of this combination of abnormalities in a dog with clinical signs of a chronic enteropathy warrants further, more invasive diagnostic testing, such as endoscopy with mucosal biopsy or exploratory laparotomy with biopsy.

Folate and cobalamin are intrinsically linked biochemically, with most enzyme systems that rely on cobalamin as a co-factor also utilizing folate as a methyl group donor. This means that animals that are cobalamin deficient are often not utilizing folate particularly efficiently, which can result in accumulation of folate in the circulation. When the low cobalamin is detected and supplementation begins, it is common for serum folate concentrations to drop quite markedly, in some cases folate drops low enough to suggest the presence of duodenal mucosal disease.

The clinical significance of elevated cobalamin concentrations remains unclear. At least one publication in the veterinary literature has associated high cobalamin concentrations with some hepatic and neoplastic diseases in cats,[12] similar data are lacking for dogs.

Common patterns of results and interpretation for cobalamin and folate

The table below summarizes the common patterns of results that may be detected when measuring serum cobalamin and folate concentrations in dogs and cats with gastrointestinal disease. It is important to remember that these tests have high specificities due to the very localized absorption sites, but they have relatively low sensitivities and thus these tests **cannot** be used to rule out the presence of small intestinal mucosal disease.

Cobalamin	Folate	Potential DDx
Low	Low	Diffuse SI mucosal diseases: Infiltrative (IBD, LSA) Structural (lymphangectasia, Short Bowel Syndrome)
Low	High	Disturbed intestinal flora: "SIBO". Diffuse SI mucosal Exocrine Pancreatic Insufficiency (particularly in cats), check [TLI]
Low	Normal	Distal SI disease (infiltrative or structural) MOST LIKELY Abnormal bacterial population/dysbiosis Exocrine Pancreatic Insufficiency (particularly in cats), check [TLI]
High	Normal	Possible association with hepatic and neoplastic disease in cats, consider iatrogenic sources, coprophagia
Normal	High	Intestinal dysbiosis if compatible signs Possibly no significance, consider iatrogenic sources, coprophagia

Indications for supplementation

As well as acting as a marker for intestinal mucosal disease, there is an increasing body of evidence that cobalamin deficiency, which can manifest with serum cobalamin concentrations in low end of the normal range for both dogs and cats,[13,14] is associated with poorer response to therapy and poorer prognosis in a variety of diseases.[15-17] While a full discussion of cobalamin supplementation dosing and schedules is outside the scope of these notes, a substantial amount of information is available from the GI Lab at Texas A&M website, at: http://vetmed.tamu.edu/gilab/research/cobalamin-information

Low serum folate concentrations will also often prompt supplementation, and anecdotally there does appear to be a link between low serum folate and poorer response to therapy, but objective data regarding thresholds for supplementation and doses required are lacking at this time. The author typically recommends folic acid supplementation, 5-10 µg/kg *per os* daily for dogs and cats with serum folate concentrations <4.5µg/L. Additionally, animals with low normal serum folate and subnormal cobalamin concentrations receive folate supplementation preemptively, due to the common occurrence of low folate following cobalamin supplementation.

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