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VCA Clinical Consults

What's Your Diagnosis?

IBD OR MORE?

Utilizing Second Opinions and Advanced Diagnostic Testing

Donna J. Spector , DVM, DACVIM, Internal Medicine

PATIENT HISTORY

SIGNALMENT: "Paulie" is a 12 year old neutered male domestic shorthair cat. Weight 11.3 lb/5.14 kg.

PERTINENT PAST HISTORY: "Paulie" has generally been a healthy cat. He has outdoor access to a fenced backyard on a daily basis. "Paulie" has historically been a hunter, although his owners report his hunting activity has slowed considerably within the last year and they have never witnessed him to consume prey. "Paulie" eats adult commercial canned and dry cat food. He is current on recommended vaccinations and receives monthly preventives.

"Paulie" has a history of dental disease with multiple extractions for resorptive odontoclastic lesions (FORLs). He has sustained multiple bite wounds which required drainage procedures and antibiotic usage in previous years. In 2015, "Paulie" was diagnosed with hyperthyroidism (with clinical signs of weight loss, hyperactivity, vocalization and voracious appetite) and has been stable receiving methimazole (5 mg orally every 12 hours) for several years.

CURRENT HISTORY: "Paulie" presented to his regular veterinarian for evaluation of ongoing weight loss, intermittent vomiting and occasional diarrhea for several weeks' duration in June 2018. His owners were also concerned about the increased amount of water he had been drinking in the past six months. See **Table 1** for additional pertinent history. This table summarizes "Paulie's" previous two years of visits to his primary care veterinarian AFTER his hyperthyroidism had been well controlled based on low to low-normal serum T4 concentrations and resolution of polyphagia, hyperactivity and vocalization.

TABLE 1.
ADDITIONAL PERTINENT HISTORY

May 2016	**13.6 pounds**	Moderately decreased appetite, lethargic, occasional vomiting
October 2016	**13.0 pounds**	Vomiting
January 2017	**12.6 pounds**	Not eating, diarrhea for several days, seemed thinner to owner
May 2017	**12.2 pounds**	Not eating, diarrhea for several days, lethargic
April 2018	**11.7 pounds**	Drinking more, losing weight, vomiting more than "normal"

Clinical Note: I feel it is important to highlight the chronic nature of "Paulie's" history as I am frequently referred patients like "Paulie" and this type of pattern can be traced over many months to sometimes years. Despite excellent control of his hyperthyroidism (and no other apparent abnormalities on bloodwork during these visits), "Paulie" continued to exhibit intermittent gastrointestinal clinical signs as well as ongoing persistent weight loss. While each of these individual episodes of gastrointestinal distress would resolve with supportive care, a distinct pattern of recurrence with ongoing weight loss was present. This should be addressed as soon as it is noted by a primary care veterinarian with an appropriate diagnostic workup pursued in a timely manner.

After further evaluation of his chronic history of weight loss and episodic clinical signs, "Paulie's" veterinarian recommended a minimum database to include a CBC, chemistry panel, T4, and urinalysis. While all of "Paulie's" laboratory values technically fell within normal reference ranges, the values of note were as follows: T4 0.8 ug/dL (reference range: 0.8-4.7 ug/dL), BUN 22 mg/dL (reference range: 16-37 mg/dL), creatinine 1.8 mg/dL (reference range: 0.9-2.5 mg/dL), albumin 2.6 g/dL (reference range: 2.6-3.9 g/dL), usg 1.021, negative protein/sediment. ***Clinical Note: IRIS staging guidelines recognize renal azotemia may begin with blood creatinine concentrations lower than most reference ranges and is likely closer to 1.6 mg/dL in the cat. With the creatinine of 1.8 mg/dL and sub-par urine specific gravity, "Paulie's" owner was counselled he most likely had early renal disease (IRIS stage 2) as the cause for his increased water consumption. However, this degree of renal disease is not a likely etiology of his magnitude of weight loss or ongoing clinical signs of vomiting.***

As hyperthyroidism or other obvious metabolic diseases were not overtly the cause for "Paulie's" ongoing weight loss and clinical signs, he was referred for abdominal ultrasound imaging to more thoroughly evaluate for gastrointestinal disease. He was also empirically dewormed given his outdoor and hunting status. See **Table 2** for the ultrasonographic findings.

Given the overt abnormalities noted in the spleen, a fine needle aspirate of "Paulie's" spleen was performed at the time of his ultrasound examination. See **Table 3** for cytologic results.

The predominance of small lymphocytes (lymphocytic hyperplasia) within the spleen was very concerning for the possibility of small cell lymphoma within the spleen or even more likely within the gastrointestinal tract as this is a very common site of origin – especially in a cat with "Paulie's" history and ultrasonographic findings of a diffusely thickened GI tract. The following options were discussed with the owner:

- PARR (PCR for antigen receptor rearrangements) on the splenic cytology to determine if the spleen contained a neoplastic population of lymphocytes. ***Clinical Note: The spleen could contain a reactive population of lymphocytes while the neoplastic population could reside within the gastrointestinal tract and be missed with this approach.***
- Endoscopy to address the diffuse gastrointestinal thickening identified on ultrasound. ***Clinical Note: If the gastrointestinal biopsies were to return unclear or there was still possible concern for lymphoma, PARR could be performed on the histopathology samples.***
- Abdominal exploratory for splenectomy and full thickness gastrointestinal biopsies.

The owner opted for endoscopic biopsies. This was felt to be an excellent choice for "Paulie" especially with the option of PARR molecular diagnostics available should it be necessary to help differentiate between a difficult or ambiguous case of IBD versus small cell lymphoma.

ADDITIONAL DIAGNOSTICS

"Paulie" underwent standard preparation for anesthesia and upper and lower endoscopy. Endoscopic examination revealed a pale roughened duodenum but otherwise normal esophagus, stomach, colon and ileocolic valve. ***Clinical Note: Although "Paulie" had no specific large bowel clinical signs***

TABLE 2.
"PAULIE'S" ABDOMINAL ULTRASOUND REPORT

ULTRASONOGRAPHIC FINDINGS: The liver is normal in size and shape. The parenchyma is within normal limits for echogenicity and echotexture. No focal lesions are noted. The gallbladder is small and normal. The spleen is within normal limits for size and shape, however, the splenic parenchyma has a diffusely mottled to moth-eaten appearance. Kidneys are slightly reduced in size and have moderately hyperechoic cortices. No pelvic dilation is noted. The urinary bladder is moderately distended with anechoic urine. Bladder wall is normal. The adrenal glands are normal bilaterally. The gastric wall is normal in thickness and layering. The small intestine is diffusely thickened up to 0.36 cm with normal layering. The colon is normal. There are a few mildly enlarged mesenteric lymph nodes noted. The pancreas appears normal. There is no ascites appreciable.

ULTRASONOGRAPHIC IMPRESSIONS:

- Diffuse enteropathy – rule out infiltrative disease (IBD vs LSA vs other)
- Mild mesenteric lymphadenopathy – rule out reactive vs infiltrative / neoplastic disease
- Mottled splenic architecture – rule out reactive vs infiltrative / neoplastic disease
- Mild chronic renal changes

TABLE 3.
"PAULIE'S" SPLENIC CYTOLOGY RESULTS

Microscopic Description: The smears are hemodiluted and highly cellular. Intact nucleated cells are comprised of a majority of lymphocytes, with far fewer plasma cells. The lymphocyte population is comprised of approximately 60-70% small lymphocytes, with a robust minority population of intermediate lymphocytes approaching 40% in some areas. Lymphocytes have mature chromatin. No infectious agents are observed.

Microscopic Diagnosis: Most consistent with moderate reactive lymphoid hyperplasia

Comments: Lymphocytes are numerous with a modest majority of small cells. Intermediate lymphocytes approach 40% in some areas but are a visible minority overall. There are sporadic plasma cells. The findings are most supportive of reactive lymphoid hyperplasia. **Please correlate with any detected lymphadenopathy which could possibly be suggestive for emergent lymphoma (the disease typically presents in the feline GI tract +/- associated lymphadenopathy).**

(i.e., hematochezia, mucus, tenesmus, and/or urgency), colonoscopy was performed in addition to gastroduodenoscopy as it is the only way to obtain ileal biopsies; which is an important part of complete gastrointestinal evaluation for affected cats. Multiple biopsies were taken in all gastrointestinal sections. Blind ileal biopsies were obtained through the ileocolic valve. See **Table 4** for histopathology results.

The original histologic diagnosis was mild to moderate lymphocytic enteritis **(see Table 4).** ***Clinical Note: Given "Paulie's" duration and progression of clinical signs as well as the severity of his weight loss, I was concerned about a more severe disorder. There was also a brief mention of epitheliotropism on his histopathology which is a classic feature of lymphoma. As such, I requested a second opinion from the reference laboratory.***

Do not hesitate to request a second opinion from your reference laboratory – especially if your patient's clinical picture does not seem to fit with the reported histopathologic diagnosis! Most pathologists welcome the feedback and are more than happy to obtain re-cuts of tissue, ask colleagues to review cases and strive to give you the best possible answer.

Two fairly recent studies in cats and dogs with cancer reveal second-opinion histopathology helps avoid unnecessary procedures, costs and helps optimize therapy. One study reported diagnostic agreement between first and second opinion in 52% of cases. Twenty-nine percent (29%) of cases had partial diagnostic disagreement; most often a change in grade, tumor subtype or margin status. Nineteen percent (19%) had complete diagnostic disagreement including a change in cell of origin or a change from benign to malignant. Minor disagreements (which would not affect treatment or prognosis) were present in 21% of cases. Major disagreements (which would affect either treatment or prognosis) were present in 37% of cases. Costs of ideal staging and treatment recommendations were considerably different between first and second opinions.

Two additional pathologists reviewed "Paulie's" case and concluded the histopathologic assessment was accurate in all sections with the exception of the ileum. See **Table 5** for the amended ileal histopathology description.

TABLE 4.
"PAULIE'S" GASTROINTESTINAL HISTOPATHOLOGY

STOMACH

Microscopic Description: The propria contains minimally increased numbers of lymphocytes, plasma cells and occasional eosinophils and macrophages. Minimal superficial propria edema is present.

Interpretation: Minimally increased propria lymphocytes with minimal edema.

Comments: The gastric changes are minimal and could represent the consequence (rather than the cause) of the vomiting and/or extension of the more significant small intestinal pathology. There was no evidence of neoplasia.

DUODENUM and ILEUM

Microscopic Description: The lamina propria contains mildly to moderately increased numbers of small lymphocytes with smaller numbers of plasma cells and occasional eosinophils and macrophages. Multifocally, the small lymphocytic component exhibits mild epitheliotropism.

Interpretation: Mild/moderate lymphocytic enteritis.

Comments: Changes in the duodenum and ileum are compatible with a mild case of chronic inflammatory bowel disease (IBD). IBD is a disorder characterized by persistent or recurrent gastrointestinal signs with histological evidence of inflammation. The inflammation can be lymphocytic, lymphoplasmacytic, eosinophilic, or granulomatous in character. It is believed there is a breakdown of immunologic tolerance to luminal antigens (bacteria, bacterial products, dietary components). The breakdown in tolerance may be associated with disruption of the mucosal barrier, dysregulation of the immune system, and/or disturbances in the gut luminal microenvironment. Definitive interpretation of true idiopathic IBD requires exclusion of recognized parasitic, allergic, and/or infectious causes of inflammation.

TABLE 5.
AMENDED ILEUM HISTOPATHOLOGY

ILEUM ADDENDUM

Microscopic Description: The lamina propria contains mildly to moderately increased numbers of small lymphocytes with smaller numbers of plasma cells and occasional eosinophils and macrophages. Multifocally, the small lymphocytic component exhibits moderate epitheliotropism with occasional intraepithelial aggregates of 4-5 cells. **Interpretation: Mild/moderate lymphocytic enteritis, concerning for emerging small cell epitheliotropic lymphoma.**

Comments: While most features appear to be compatible with a mild case of IBD, there is increased epitheliotropism, with occasional intraepithelial aggregates of 4-5 small lymphocytes. This feature is highly concerning for the incipient stage of a small cell epitheliotropic lymphoma.

"Paulie's" ileal biopsies exhibited features of moderate epitheliotropism with intra-epithelial aggregates of lymphocytes - which are features concerning for small cell lymphoma. PARR testing was advised based on these features to aid in resolving this more ambiguous case. ***Clinical Note: PARR testing can be performed on formalin fixed tissues at some reference laboratories. It is always best to contact your reference laboratory to obtain more information about this specialized testing before sample submission.***

"Paulie's" clonality assay returned as positive for T-cell gastrointestinal lymphoma. See **Table 6** for these PARR results. ***Clinical Note: "Paulie's" case highlights the importance of including colonoscopy for ileal biopsies in cats presenting for diffuse gastrointestinal disease. If ileal biopsies had not been done, the diagnosis of lymphoma would have been missed in this patient!***

TABLE 6.
"PAULIE'S" PARR RESULTS

IGH Locus (B cell) –
IGH-FR2: no significant amplification
IGH-FR3: no significant amplification

TCRG Locus (T cell) – Clonal in polyclonal background

Diagnosis: Small intestine – lymphoma, presumed T-cell lymphoma, enteropathy-associated type II

Comments: Clonality testing targeting the feline T-cell receptor gamma (TCRG) locus revealed multiple clonal rearrangements in a polyclonal background. Clonality testing targeting the feline immunoglobulin heavy chain locus (IGH) revealed nothing significant with two different primer sets (FR2/FR3).

In conjunction with the clinical and histological findings, these results are consistent with a diagnosis of intestinal lymphoma. The neoplasm is most likely of T cell origin. The confidence in this diagnosis is high.

DIAGNOSIS

IRIS stage 2 CKD
Splenic lymphoid hyperplasia
Moderate lymphocytic IBD
T-cell gastrointestinal lymphoma

DISCUSSION

Detailed discussion of feline IBD, gastrointestinal lymphoma and PARR testing are beyond the scope of this article and the reader is referred to the listed references.

Clonality Assays

Any diagnostic test with the ability to demonstrate a group of cells derived from a single clone can be considered a clonality assay. The clonality assay used most commonly in veterinary medicine to aid in the diagnosis of lymphoma in cytologically and histologically ambiguous cases is called PARR (PCR for antigen receptor rearrangements).

Unique genes found in individual B-cells are called Ig genes and those found in T-cells are called T-cell receptor genes. The portion of these genes that encodes the antigen-binding region is the portion that varies between cells, both in size and sequence. Once a B-cell or T-cell is mature and divides in response to antigenic stimulation, the unique Ig and T-cell receptor genes are passed on to the daughter cells.

In the course of a normal immune response to a pathogen, B-cells and T-cells are activated, undergo clonal expansion, and eventually die, leaving behind only a small number of residual memory cells. On the other hand, when a cell becomes neoplastic, it is no longer subjected to these normal growth controls, and can expand significantly more than the cells during an immune response. Therefore, if one can establish the majority of cells in a particular collection of lymphocytes having the same Ig or T-cell receptor gene, it is most likely these cells are neoplastic rather than reactive.

Clonality assays first isolate DNA from cells suspected to be neoplastic, then use PCR primers directed at the conserved regions of T-cell receptor or Ig genes that flank the hypervariable regions of these genes to amplify the variable regions. The PCR products are then separated by size. The presence of a single-sized PCR product is indicative of clonality, whereas the presence of multiple PCR products supports a reactive process.

In practice, the PARR assay is most commonly used to aid in distinguishing reactive (polyclonal) from neoplastic (monoclonal) lymphocytes when these distinctions are difficult to make with other means such as cytology or histology. ***Clinical Note: PARR is an ideal test to perform on a patient such as "Paulie" with lymphocytic hyperplasia in the spleen (on splenic aspiration) or on gastrointestinal histopathology which revealed some lymphocytic epitheliotropism but a confirmed diagnosis of lymphoma could not be made.***

I find I utilize PARR testing quite frequently on histopathology samples from my feline IBD patients. The distinction between lymphocytic inflammatory disease and insidious or emerging small cell lymphoma is often not clear clinically or histologically and in fact may represent a continuum of dysregulation of lymphocyte proliferation. Clonality testing often proves very clinically useful in these types of cases in differentiating an inflammatory response from an emerging lymphoma.

There are several applications that are not appropriate for PARR. PARR is not intended as a first-line diagnostic procedure, and in fact, is often not necessary. For example, although PARR testing can provide lineage information (B-cell vs T-cell) when it is positive, if the diagnosis of lymphoma or leukemia is unambiguous, flow cytometry, immunocytochemistry, or immunohistochemistry are better tests to determine the phenotype because more information can be obtained. Therefore, PARR testing is less useful for confirmed cases of lymphoma. These assays can, however, provide a wealth of clinically useful information in resolving ambiguous cases.

"PAULIE'S" TREATMENT

Cobalamin and folate levels were performed to evaluate for malabsorption. "Paulie's" cobalamin levels were low at 215 pg/mL (reference range 290-1500 pg/mL) and he was started on weekly supplementation. ***Clinical Note: All cats undergoing diagnostic assessment for small intestinal disease should have serum cobalamin and folate concentrations measured. Failure to recognize and treat hypocobalaminemia often results in incomplete resolution of gastrointestinal clinical signs, despite appropriate therapy for the primary disease.***

"Paulie" was prescribed prednisolone (5 mg PO every 12 hours) and chlorambucil for management of his IBD and gastrointestinal lymphoma. There are many protocols for chlorambucil administration but his was administered in a pulse fashion. He was scheduled to receive 15 mg/m2 of chlorambucil orally daily for 4 consecutive days; repeated every 3 weeks. A CBC will be

monitored initially after 2 weeks and then monthly for 3 months. Once stable and if he is tolerating therapy well, a recheck every 3 months is often adequate. With "Paulie's" renal disease, additional close monitoring of his azotemia and blood pressure are warranted while on prednisolone and chlorambucil therapy.

Combination chlorambucil and prednisolone chemotherapy is the treatment of choice for lymphocytic GI lymphoma. This is an outpatient protocol which can easily be administered by owners at home. The rationale for using this less-intensive chemotherapy regimen is that the slowly dividing low-grade lymphocytic lymphoma

warrants treatment with slow alkylating cytotoxic agents more commonly used for indolent neoplasms. The slow alkylating agents (e.g., chlorambucil) are inherently less toxic and tend to be well tolerated by most cats.

It is crucial to define the grade of lymphoma (high-grade versus low-grade) before prognosticating for clients. Low-grade intestinal lymphoma has a much less aggressive biologic behavior and carries a favorable long-term prognosis using a fairly simple, relatively non-toxic chemotherapeutic protocol. The prognosis for treated cats is approximately 24 months, however, many cats achieve a long-lasting clinical remission and do well for years.

REFERENCES

Vail, David M. **Hematopoietic Tumors,** Chapter 324. The Textbook of Veterinary Internal Medicine, Ettinger and Feldman editors, 7th edition, Elsevier, St. Louis, 2017.

Regan, RC, Rassnick KM, Malone EK, McDonough SP. **A prospective evaluation of the impact of second-opinion histopathology on diagnostic testing, cost and treatment in dogs and cats with cancer.** Vet Comp Oncol. June 2015; 13(2): 106-16.

Regan RC, Rassnick KM, Balkman CE, et al. **Comparison of first-opinion and second-opinion histopathology from dogs and cats with cancer: 430 cases (2001-2008).** Vet Comp Oncol. March 2010; 8(1): 1-10.

Avery Anne. **Molecular Diagnostics of Hematologic Malignancies.** Topics in Companion Animal Medicine Volume 24, Number 3, August 2009.

Stein TJ, Pellin M, Steinberg H, et al. **Treatment of feline gastrointestinal small cell lymphoma with chlorambucil and glucocorticoids.** J Am Anim Hosp Assoc 2007 Vol 46 (6) pp. 413-7.

CLINICAL ASSESSMENT

1 Which of the following terms are often used interchangeably to describe gastrointestinal lymphoma in cats?

- **a.** Well differentiated
- **b.** Lymphocytic
- **c.** Small cell
- **d.** Low grade
- **e.** All of the above

2 Gastrointestinal lymphoma in cats is usually lymphocytic and often T-cell in cell type and carries a very poor prognosis. **True or False?**

3 Injectable chemotherapy protocols (like CHOP) are preferred over oral chemotherapy protocols for lymphocytic gastrointestinal lymphoma. **True or False?**

4 The PARR assay is:

- **a.** Capable of distinguishing reactive from neoplastic lymphocytes
- **b.** Helpful in resolving ambiguous cases of cytology/histology
- **c.** Not intended as a first line diagnostic test
- **d.** Capable of providing cell lineage information
- **e.** All of the above

5 Cats undergoing endoscopy for diffuse gastrointestinal disease need only have gastroduodenoscopy performed. **True or False?**

6 Histopathologic features of gastrointestinal lymphocytic lymphoma include:

- **a.** Epitheliotropism
- **b.** Intraepithelial lymphocyte aggregates
- **c.** No accompanying changes in crypt or villus enterocytes
- **d.** No mucosal congestion, edema or fibrosis
- **e.** All of the above

7 Feline IBD and gastrointestinal lymphocytic lymphoma may represent a continuum of lymphocyte dysregulation and disease progression. **True or False?**

8 Lymphocytic hyperplasia in the spleen or mesenteric lymph node should raise the index of suspicion for possible small cell/lymphocytic lymphoma within the gastrointestinal tract. **True or False?**

9 The following is/are indication(s) to obtain second opinions on histopathology reports:

- **a.** Unexpected results
- **b.** Clinical picture of patient does not fit histopathologic results
- **c.** Avoid unnecessary procedures and expense
- **d.** Optimize therapy
- **e.** All of the above

CLINICAL ASSESSMENT ANSWERS:

1. **e. All of the above.** The most common form of gastrointestinal lymphoma in the cat is a form of low-grade, well-differentiated, small-cell or lymphocytic lymphoma.
2. **False.** Low-grade intestinal lymphoma has a much less aggressive biologic behavior and carries a favorable long-term prognosis. The prognosis for treated cats is approximately 24 months, however, many cats achieve a long-lasting clinical remission and do well for years.
3. **False.** Slow oral alkylating cytotoxic chemotherapy protocols are preferred over more rapidly cytotoxic injectable protocols (like CHOP) due to the slowly dividing nature of lymphocytic gastrointestinal lymphoma.
4. **e. all of the above.** The PARR assay is best for distinguishing reactive from neoplastic lymphocyte populations and being able to resolve ambiguous cases of cytology and histology. Although it can provide cell lineage information (B-cell versus T-cell) it is not the best assay for this.
5. **False.** Even in the absence of large bowel clinical signs, colonoscopy is important in order to obtain ileal biopsies. In "Paulie", the ileum was the only gastrointestinal section with histopathologic evidence of lymphoma. Had ileal biopsies not been procured, the diagnosis would have been missed altogether!
6. **e. all of the above**
7. **True.** IBD and lymphoma are often difficult to distinguish clinically and/or histologically. Clinically cats with lymphoma tend to exhibit more profound weight loss and signs of malabsorption. PARR testing gives us a great new tool to assist in the diagnosis of ambiguous cases when cytology or histology is unclear.
8. **True.** Lymphocytic lymphoma typically presents in the feline gastrointestinal tract but often has associated lymphadenopathy or splenomegaly. These organs may be infiltrated with either reactive or neoplastic small lymphoid cell populations.
9. **e. All of the above.** Second opinions on histopathologic samples should be readily sought in cats and dogs with cancer to help avoid unnecessary procedures and to help optimize therapy. One recent study reported diagnostic agreement between first and second opinion in 52% of cases. However, 29% of cases had partial diagnostic disagreement and 19% had complete diagnostic disagreement including a change in cell of origin or a change from benign to malignant. Minor disagreements (which would not affect treatment or prognosis) were present in 21% of cases. Major disagreements (which would affect either treatment or prognosis) were present in 37% of cases.