CREATING CLARITY

Lymphoma: getting to grips with confusing histopathology diagnostics

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Disclosure:

I’m an IDEXX employee and the information contained herein is intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical presentation, and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions, and cautions. Diagnosis and treatment decisions are the ultimate responsibility of the primary care veterinarian.

What can be a confusing diagnosis?

1) Diagnosis of lymphoma and not knowing what to do next

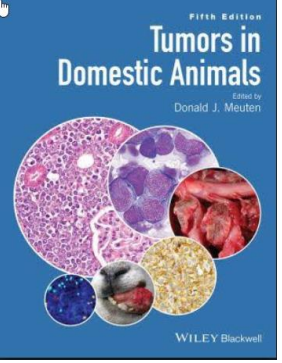
2) Diagnosis of probable lymphoma and not understanding the ancillary testing results:

   - clonality testing (PARR) and/or
   - immunohistochemistry (IHC)

THERE ARE SOME CONFUSING HISTOPATHOLOGIC DIAGNOSTICS BUT THERE SHOULD NOT BE ANY CONFUSING HISTOPATHOLOGY REPORTS

The ultimate goal with lymphoma diagnostics today is to provide sufficient information on the lymphoma involved to be able to offer today’s most specific and advanced treatment related to the entity identified.

Humans >>> Dogs > Cats > Horses > other animals



Lymphoma WHO classification applied in animals, mainly in dogs

Meuten DJ, ed. ***Tumors in Domestic Animals, 5th Edition*** Wiley Blackwell, Hoboken, New Jersey, USA, 2016, 1008 pp

B-cell neoplasms

Precursors B-cell neoplasms
- Lymphoblastic leukemia / lymphoma

Mature (peripheral) B-cell neoplasms
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- Prolymphocytic leukemia
- Lymphoplasmacytic lymphoma
- Plasmablastic lymphoma
- Mantle cell lymphoma (MCL)
- Follicular lymphoma
- Diffuse large B-cell lymphoma (DLBCL) **50%**
  - Subtypes: T-cell-rich large B-cell; primary mediastinal (thymic)
- Angiocentric B-cell lymphoma (lymphomatoid granulomatous)
- Marginal zone lymphoma (MZL) **5-10%**
  - Nodal, splenic, extranodal MZL of mucosa-associated lymphoid tissue type (MALT)

Burkitt's lymphoma / Burkitt's cell leukemia

Provisional entity: high-grade B-cell lymphoma Burkitt's-like

Plasma cell myeloma

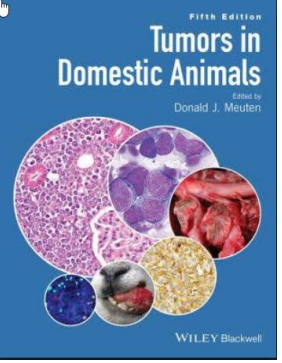
Plasmacytoma

T-cell and putative NL-cell neoplasms

Precursor T-cell neoplasm
- Lymphoblastic lymphoma (LBL) / leukemia **3-5%**

Mature (peripheral) T-cell and NK-cell neoplasms
- Chronic lymphocytic leukemia (CLL) / small cell lymphoma (SLL)
- Prolymphocytic leukemia
- Large granular lymphocytic (LGL) leukemia / lymphoma
- T-zone lymphoma (TZL), nodal **3-15%**
- Intestinal T-cell lymphoma (enteropathy associated)
- Hepatosplenic γδ T-cell lymphoma
- Mycosis fungoides / Sézary syndrome
- Intravascular lymphoma (angiocentric)
- Subcutaneous panniculitis-like T-cell lymphoma
- Angioimmunoblastic T-cell lymphoma
- Aggressive natural killer (NK)-cell leukemia / lymphoma
- Adult T-cell lymphoma / leukemia
- Anaplastic large cell lymphoma; cutaneous and systemic
- Peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) **15%**

- The five tumours marked with a red percentage account for ~ 80% of canine lymphomas.
- T-zone lymphoma is not part of the human WHO classification but it is listed here because it is one of the most common lymphomas in dogs.
- Peripheral T-cell lymphomas not otherwise specified (PTNOS) are those that are not presently specified to a specific subtype



Lymphoma WHO classification applied in cats

Meuten DJ, ed. ***Tumors in Domestic Animals, 5th Edition*** Wiley Blackwell, Hoboken, New Jersey, USA, 2016, 1008 pp

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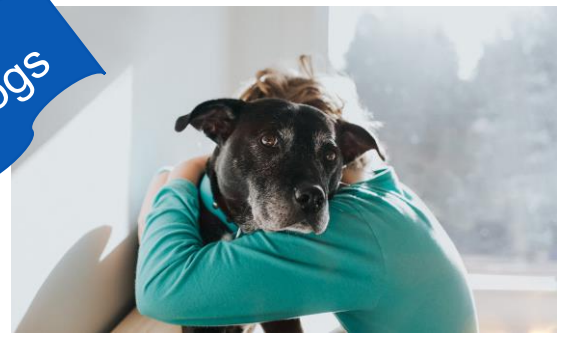
- WHO classification is less of use in cats (at least today).
- Lymphoma forms are different: mainly gastrointestinal (~50% of feline lymphoma cases) mediastinal, nasal.
- Response to different treatments have been less studied.
- Most common WHO lymphoma subtype in cats is the T-cell-rich large B-cell lymphoma, also called Hodgkin's-like lymphoma (~10% of all feline lymphomas typically presenting as one or a fee adjacent enlarged cervical lymph nodes.

Lymphoma histopathology reporting today

Dogs



Lymphoma classified as a WHO entity with no additional testing

Cutaneous epitheliotropic lymphoma
Follicular lymphoma
Marginal zone lymphoma (MZL)
Mantle cell lymphoma (MCL)
(T-zone lymphoma)
(Intestinal small T-cell lymphoma)

Lymphoma + suggestion of most likely WHO entity(ies)

e.g., Lymphoma, high grade, most likely diffuse large B-cell lymphoma (DLBCL) of centroblastic subtype

↓

Immunohistochemistry
(CD3 and CD79a +/- Pax5 or CD20)

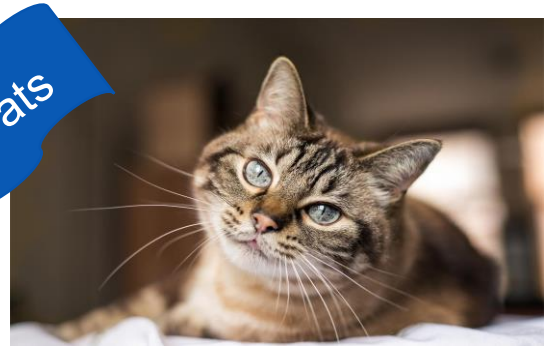
↓

T-cell or B-cell lymphoma
(1% are null-cell lymphomas and a few co-expressing both markers)

↓

WHO entity (where possible)

Cats



Lymphoma classified as a WHO entity with no additional testing

Mainly Hodgkin's like lymphoma (syn. T-cell rich large B-cell lymphoma)

Large- or small-cell lymphoma

Most common feline lymphomas

Immunohistochemistry usually not offered today as no therapy decision implications - Likely to change in the future - Discussion with oncologist always advised

IDEXX

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→ Reach out to your oncologist

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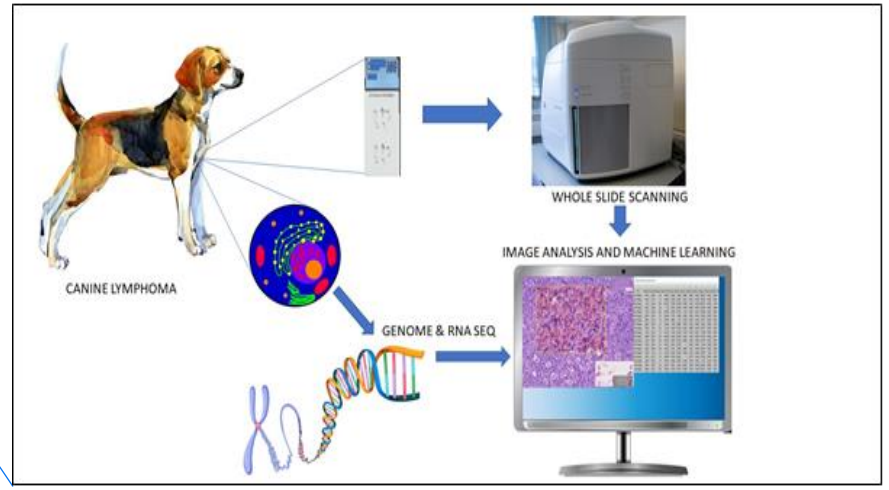
Causes of non-definitive diagnosis of lymphoma

+ Morphology of the disease (resemblance with lymphoid hyperplasia / inflammation / other round cell tumours)

+ Level of knowledge in veterinary medicine and diagnostic tools available

+ Size and tissue preservation of the sample(s)

+ Pathologist confidence / expertise (impact should be minimized)



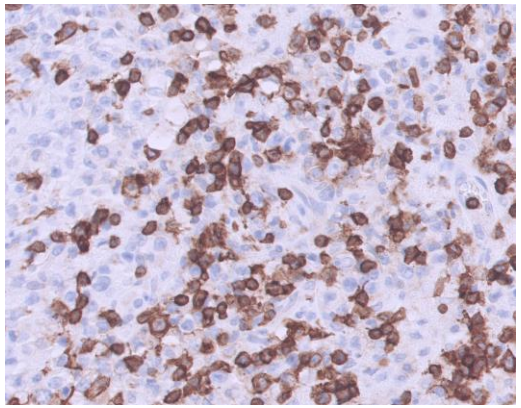
Diagnostic tools available following a diagnosis of possible / probable lymphoma at routine histopathology

+ Immunohistochemistry (IHC) :

Detection of specific antigens (proteins) in tissue samples involving the use of antibodies (markers) and visible signal.

T-cell marker: CD3

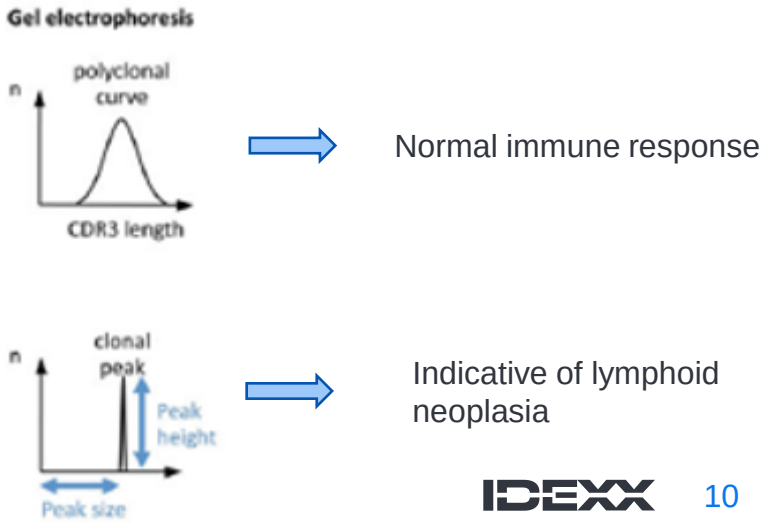
B-cell markers: CD79a, Pax5 and CD20



CD3 IHC stain – IDEXX case

+ PARR (PCR for Antigen Receptor Rearrangement) / clonality testing:

Detection of the length of amplified DNA specific regions of antigen receptor genes for both B-cells (IHG gene) and T-cells (TRG gene) by electrophoresis



Ref: Clonality Testing in Veterinary Medicine: A review with diagnostic guidelines, SM Keller et al. Vet Path 2016. Vol 53(4) 711-725.

Review

Veterinary Pathology
2016, Vol. 53(4) 711-725

DOI: 10.1177/0300985815626576
vet.sagepub.com

Clonality Testing in Veterinary Medicine: A Review With Diagnostic Guidelines

S. M. Keller[1], W. Vernau[2], and P. F. Moore[2]

Abstract

The accurate distinction of reactive and neoplastic lymphoid proliferations can present challenges. Given the different prognoses and treatment strategies, a correct diagnosis is crucial. Molecular clonality assays assess rearranged lymphocyte antigen receptor gene diversity and can help differentiate reactive from neoplastic lymphoid proliferations. Molecular clonality assays are commonly used to assess atypical, mixed, or mature lymphoid proliferations; small tissue fragments that lack architecture; and fluid samples. In addition, clonality testing can be utilized to track neoplastic clones over time or across anatomic sites. Molecular clonality assays are not stand-alone tests but useful adjuncts that follow clinical, morphologic, and immunophenotypic assessment. Even though clonality testing provides valuable information in a variety of situations, the complexities and pitfalls of this method, as well as its dependency on the experience of the interpreter, are often understated. In addition, a lack of standardized terminology, laboratory practices, and interpretational guidelines hinders the reproducibility of clonality testing across laboratories in veterinary medicine. The objectives of this review are twofold. First, the review is intended to familiarize the diagnostic pathologist or interested clinician with the concepts, potential pitfalls, and limitations of clonality testing. Second, the review strives to provide a basis for future harmonization of clonality testing in veterinary medicine by providing diagnostic guidelines.

PARR (PCR for Antigen Receptor Rearra

- Species-specific test, and exists for dogs, cats (and horse)
- Not a “perfect” test so important to know sensitivity and spe

	DOG	CAT
Sensitivity	92%	85%
Specificity	90%	90%

GD laboratories (the Netherlands)

- Not a stand-alone test, i.e. should never be interpreted ind history and ideally immunophenotyping
- Adequately fixed non-autolytic sample (to prevent false ne false positive results)
- Should NOT be used a primary lineage assignment tool / i
  1) Cross-lineage clonal receptor rearrangement report
  2) Recent study* (62 canine lymphoma cases) demons PARR agreed with IHC in approximately 69% of cas

Ref: Lymphoma immunophenotype of dogs determined by immunohistochemistry, flow cytometry, and polymerase 2013 Nov-Dec; 27(6): 1509-16.

J Vet Intern Med. 2021 Nov;35(6):2673-2684. doi: 10.1111/jvim.16231. Epub 2021 Aug 10.

Histopathologic, phenotypic, and molecular criteria to discriminate low-grade intestinal T-cell lymphoma in cats from lymphoplasmacytic enteritis

Valérie Freiche [1], Mathieu V Paulin [2], Nathalie Cordonnier [3], Hélène Huet [3], Maria-Elena Turba [4], Elizabeth Macintyre [5 6 7], Thierry-Jo Molina [7 8 9], Olivier Hermine [7 9 10], Lucile Couronné [9 11], Julie Bruneau [7 8 9]

Affiliations + expand

PMID: 34374109 PMCID: PMC8692189 DOI: 10.1111/jvim.16231

Free PMC article

Abstract

Background: Differentiation of low-grade intestinal T-cell lymphoma (LGITL) from lymphoplasmacytic enteritis (LPE) in cats is a diagnostic challenge for pathologists.

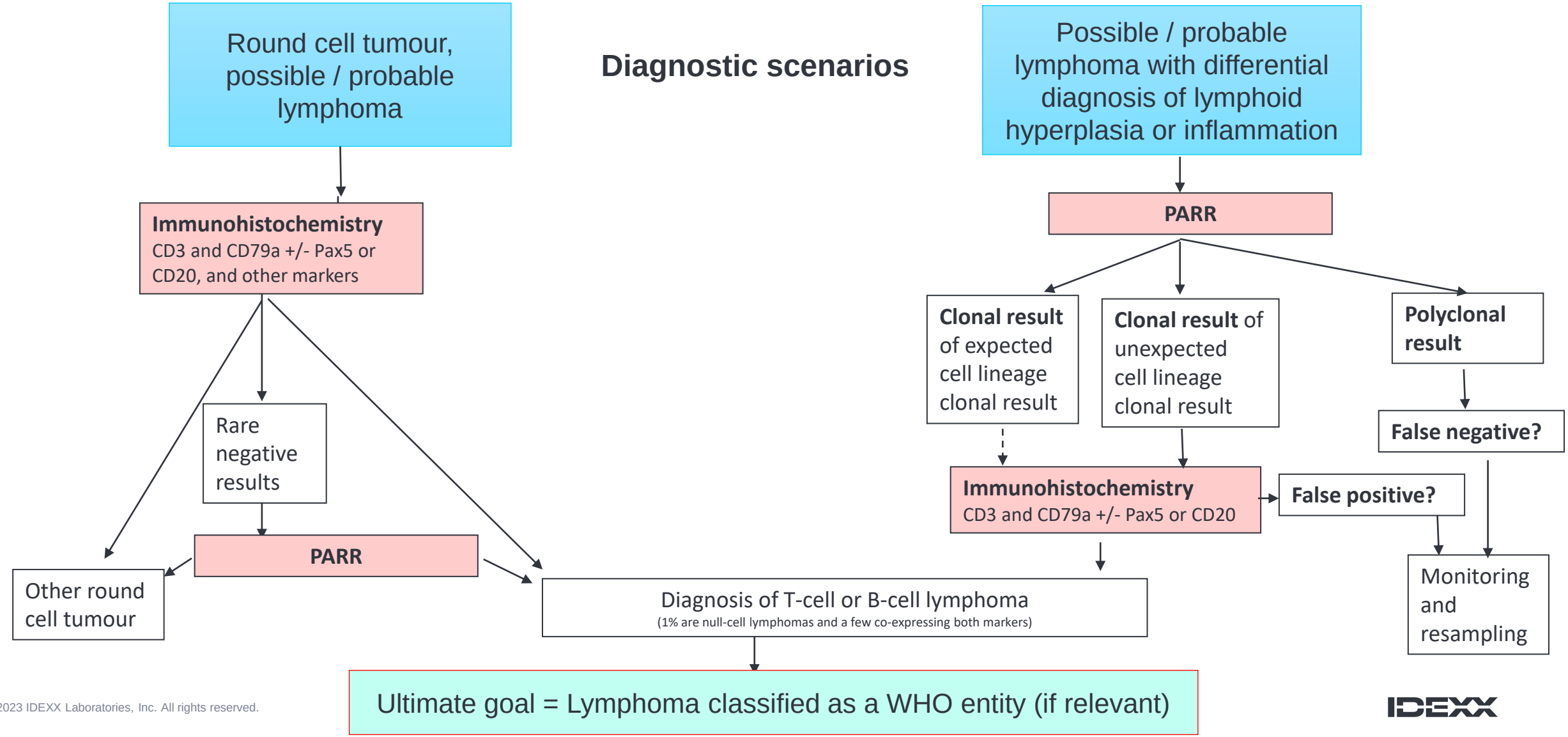
Objective: Characterize histologic, immunohistochemical, and molecular features of LGITL and LPE.

Animals: Forty-four client-owned cats, 22 diagnosed with LGITL and 22 with LPE.

Methods: Prospective, cohort study. Clinical suspicion of LGITL or LPE was based on persistent gastrointestinal signs, unresponsive to empirical treatments. All cats underwent a standardized diagnostic evaluation, including biopsy (preferentially full-thickness), and were diagnosed with LGITL or LPE after review of clinical, laboratory, sonographic, histologic, immunohistochemical, and clonality results.

Results: A monomorphic lymphocytic population (22/22, 100%) and in-depth mucosal infiltration (15/22, 68%) were hallmarks of LGITL. Epithelial patterns (nests and plaques) were significantly more frequent in LGITL (11/22, 50%) than in LPE (1/22, 5%) cases (P = .001). A CD3+ lymphocytic apical-to-basal gradient was observed in 9/22 (41%) of LGITL vs 1/22 (5%) of LPE cases (P = .004). Most LPE cases (17/18, 94%) featured marked fibrosis in the superficial part of the lamina propria. The Ki-67 20%- and 30%-thresholds discriminated between LGITL and LPE within both the epithelium (specificity >95%) and lamina propria (specificity >95%), respectively. All LGITL cases were CD3+ pSTAT3- and pSTAT5+. T-cell receptor gamma chain gene rearrangements indicated monoclonality in 86% of LGITL cases. Surprisingly, 70% of LPE cases featured monoclonality (40%) or monoclonality on a polyclonal background (30%).

Probable / possible lymphoma histopathology reporting today:



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→ Challenge / reach out to your pathologist

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References:

Victor E. Valli, Dorothee Bienzle, and Donald J. Meuten, *Tumors of the Hemolymphatic System*. In: D.J. Meuten, ed, *Tumors in Domestic Animals*, 5th edition, Wiley Blackwell, Hoboken, New Jersey, USA; 2016: 203-288.

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Happy to take questions