Lymphocytosis, Causes and Diagnostic Approach for Dogs and Cats

USEFUL DEFINITIONS:

Hematopoietic: Cells of either lymphoid (T lymphocytes, B lymphocytes and Natural killer lymphocytes) or myeloid origin (erythrocyte, monocyte, granulocyte, megakaryocyte) origin.

Lymphoproliferative disease: Denotes an expansion of neoplastic lymphocytes without distinction as to whether it is leukemia (originating in the marrow) or lymphoma (originating in the tissue).

Leukemia: A neoplastic hematopoietic cell proliferation that has originated from the marrow (i.e. the process started in the marrow as opposed to in the tissues). Additional tests such as flow cytometry and the presence of certain markers is often needed to confirm where the neoplastic proliferation started. Once modifiers (such as lymphoid or myeloid) are added it helps indicate what cell type is involved.

Acute leukemia: Acute indicates that the neoplastic cells are immature and could be either of myeloid or lymphoid origin. In these cases, morphologic features may not allow conclusive identification of cell lineage (i.e. one cannot distinguish between myeloid or lymphoid origin based on visual inspection).

Chronic leukemia: Chronic designates a neoplastic proliferation of differentiated cell types (e.g. mature lymphocytes or mature myeloid cells). Of the chronic leukemias, chronic lymphoid leukemia is the most common.

Leukemic: A term often used to indicate neoplastic cells in circulation regardless of their origin (e.g. bone marrow or tissue). However, this usage can often cause confusion.

Stage V lymphoma: Lymphoma, by definition, originates outside of the bone marrow. Neoplastic cells can enter circulation (stage V lymphoma) but this does not always indicate bone marrow infiltration. If the lymphoma is of a small origin, the cells in circulation will be small. If the lymphoma is of large cells, large cells will be found in circulation.

Reactive: Of a non-neoplastic origin.

THE OVERVIEW OF APPROACH TO LYMPHOCYTOSIS

1. First check morphology
2. Are the lymphoid cells mature or immature?
3. For mature lymphocytes, additional testing is needed (see below) to determine if the population is reactive (non-neoplastic) or neoplastic
4. If cells are immature and in high numbers they are most likely to represent acute lymphoid leukemia or stage V (cells in circulation) of lymphoma. In cases of small numbers of circulating immature cells, it is possible that the cells which appear immature are reactive (non-neoplastic).

APPROACH TO SMALL/MATURE LYMPHOCYTOSIS

If the lymphocytosis is mature, as evaluated by clinical pathologist review, several steps can be taken to identify whether the population is reactive (non-neoplastic) or is neoplastic. With small/mature lymphocytes, morphology alone is not sufficient to identify reactive versus neoplastic proliferations,

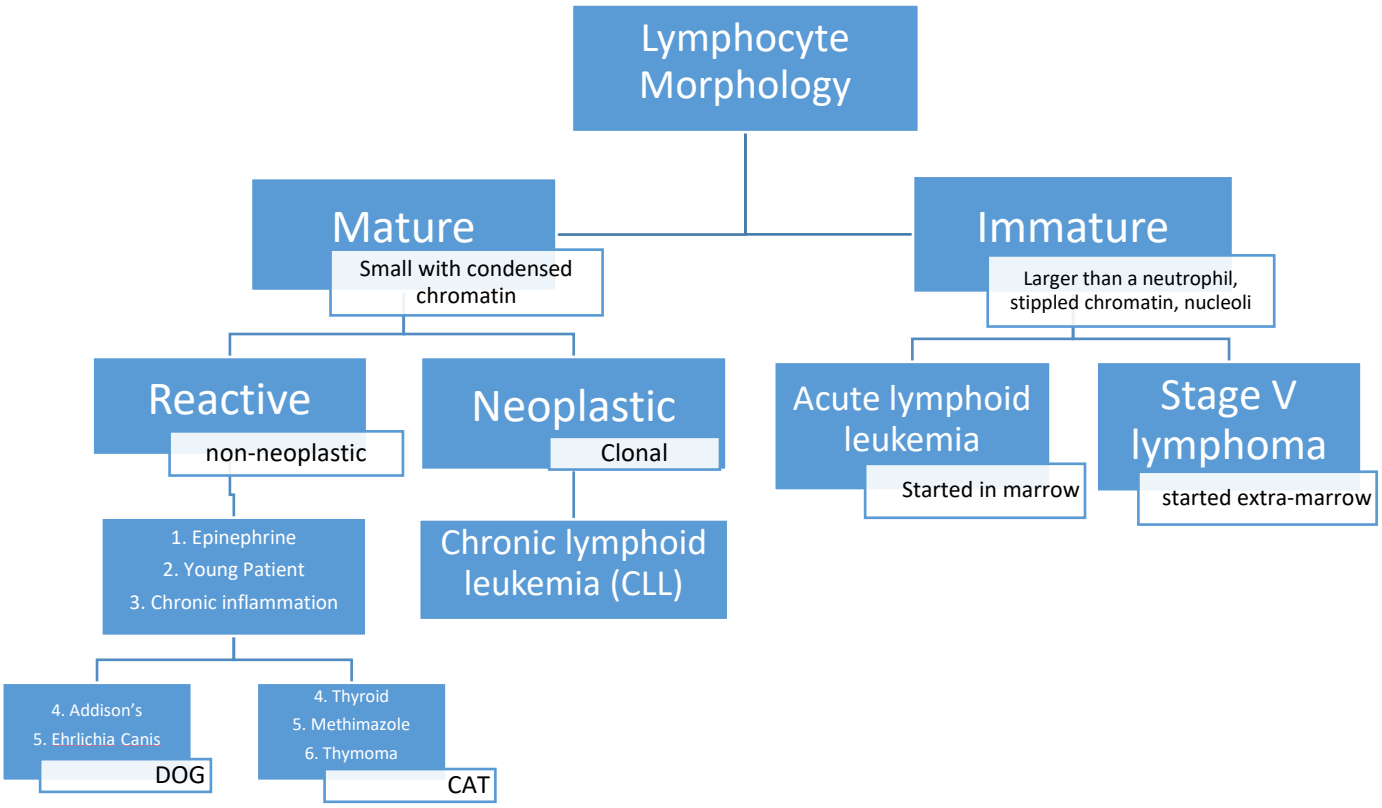
though certain magnitudes can increase suspicion of neoplasia. First, look to your blood work to see if there is support for a reactive cause (as detailed below). Whether there is good support for a reactive cause, no support for a reactive cause, or if there is only mild support, look at the magnitude of the lymphocytosis. Reactive populations do not *tend* to exceed 20,000 cells/ul in dogs and 30,000 cell/ul in cats. Thus if your numbers are higher (over the cutoff) it is more supportive of a neoplastic proliferation. If the numbers are lower than these loose cutoffs, then it could be either situation (i.e. early neoplasia or reactive) but your blood work may help you support a reactive cause in these cases.

Overall, additional steps aside from visual assessment must be taken to separate the two processes in cases of small cell lymphocytosis. These steps can include flow cytometry, establishing clonality (with PARR), identifying chromosomal abnormalities and identifying oncogenes. The first two options are more accessible in veterinary medicine and will be expanded upon in lecture. See below for an example of an algorithm on flow cytometry in canine small lymphocytosis. (Note that aspiration of organs and bone marrow is not the first test of choice when dealing with a small lymphocyte proliferation.)

If additional testing identifies that the population is reactive (non-neoplastic) there are limited causes for a small lymphocytosis. These include physiologic (epinephrine response, which should be transient), Chronic inflammation/antigenic stimulation, young patients (often due to antigenic stimulation) and hypoadrenocorticism (see below for summary points).

CAUSES (MAJOR) OF REACTIVE LYMPHOCYTOSIS (see charts below)

1. Physiologic (excitement, epinephrine-induced)
2. Chronic inflammation (antigenic stimulation)
3. Young animals
4. Dogs: Hypoadrenocorticism (Addison's disease), Ehrlichia
5. Cats: Hyperthyroidism, Methimazole therapy, Thymoma



1. CBC findings that support a lymphocytosis due to a physiologic response (epinephrine release)

• Neutrophilia (mild, mature, i.e. no left shift, normal neutrophil morphology; marginated neutrophils that have been 'washed' off of wall) • Erythrocytosis (Relative, with no evidence of dehydration e.g. splenic contraction) • Thrombocytosis (splenic contraction)

Chemistry findings (may or may not be present): • Hyperglycemia (due to epinephrine effects)

2. CBC findings that support a lymphocytosis due to the age of the animal (antigenic stimulation e.g. recent vaccination etc. or physiologic response)

• Other evidence of an inflammatory leukogram or physiologic leukogram

Chemistry findings (may or may not be present): • Hyperphosphatemia from bone growth • ± Hypercalcemia (mild) • ± Increased ALP

3. CBC findings that support a lymphocytosis due to chronic inflammation (may not have all findings concurrently)

- Neutrophilia
- Left shift
- Toxic change
- Monocytosis (inflammation, not stress given stress causes a lymphopenia)
- Anemia of chronic/inflammatory disease (less direct support)

Chemistry findings (may or may not be present):

- Hyperglobulinemia

4. CBC findings that support a lymphocytosis due hypoadrenocorticism

- ± Normal neutrophil count (in a stressed animal where you may expect a neutrophilia)
- ± Eosinophilia (due to a lack of cortisol)
- ± Mild, non-regenerative due to anemia of chronic disease or regenerative anemia (if GI bleeding)

Chemistry findings:

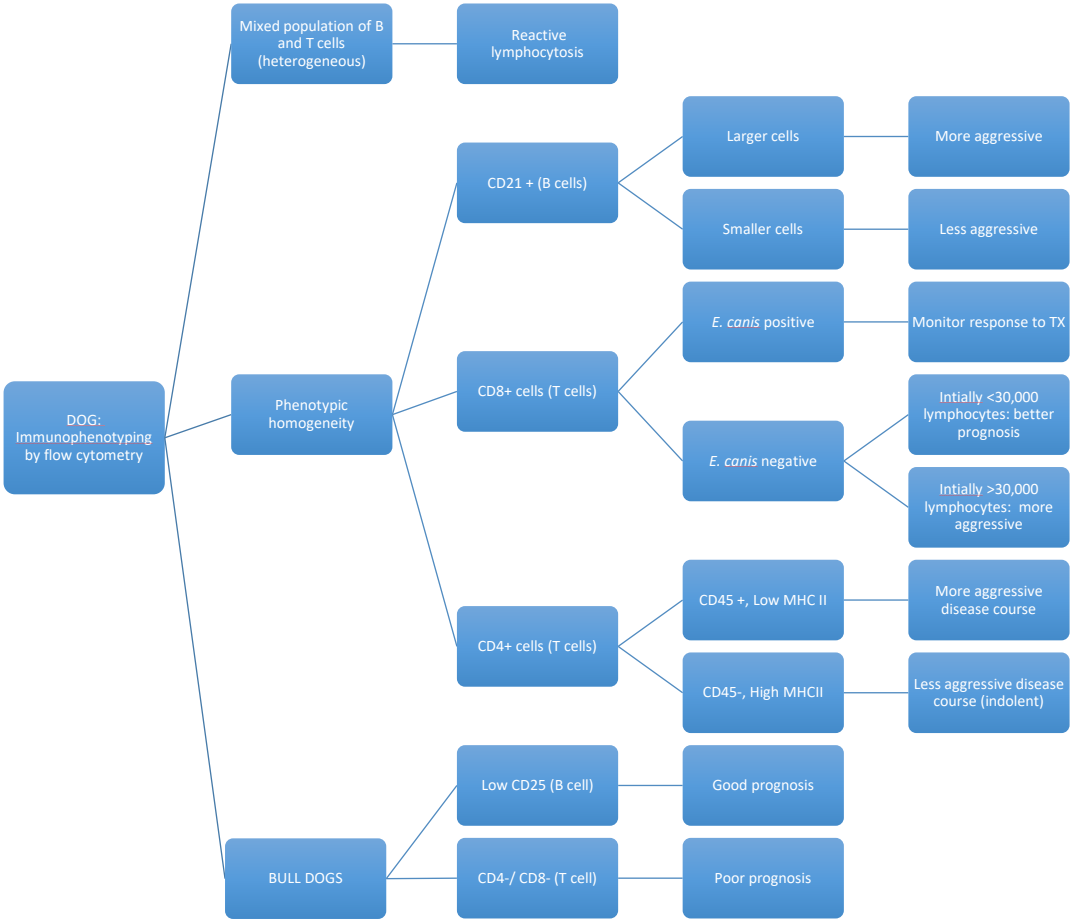
- ± Hypoglycemia (due to a lack of cortisol)
- ± Low total protein (if concurrent GI bleeding)
- ± Hypercalcemia (due to a lack of cortisol)
- ± Hyponatremia (due to a lack of aldosterone)
- ± Hyperkalemia (due to a lack of aldosterone)
- ± Pre-renal azotemia/dehydration (elevated BUN & creatinine) + poorly concentrated urine due to loss of sodium and water and a lack of urine concentrating ability, respectively (due to a lack of aldosterone)

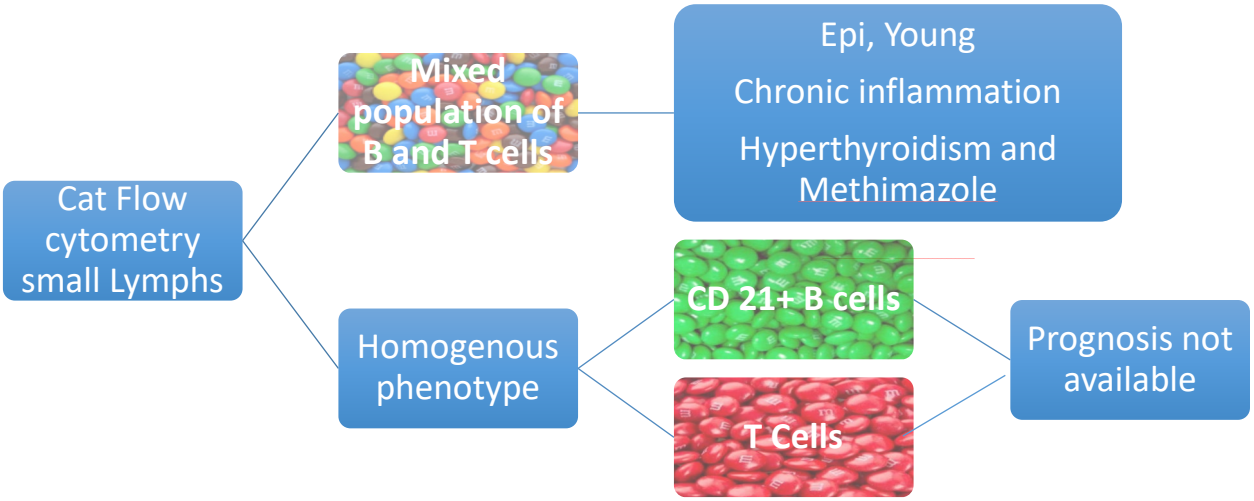
NEOPLASTIC LYMPHOCYTOSIS, CHRONIC LYMPHOCYTIC LEUKEMIA

- CLL is a heterogeneous group of lymphoproliferative neoplasms in the veterinary medicine. The word "leukemia" in the name would imply that it starts in the marrow. But this terminology was borrowed from the human disease form. For veterinary medicine when we use the term CLL we are referring to small cells in blood (but we are not necessarily certain that it started in the marrow). See below
- In people there are defined criteria for diagnosing CLL (e.g. >5,000 cells/ul, immunophenotypically atypical B cell phenotype) and it is thought to originate in marrow
- In veterinary medicine
    - CLL is generally an indolent disease but some forms are more aggressive than others
    - We can see B and T cell forms
    - One or more types of CLL can arise in spleen while others can arise from the marrow
    - Patients are often asymptomatic
    - Uncommon to have cytopenias
- In general, the lymphoid cells are smaller than neutrophils and have mature or moderately mature-appearing chromatin.
- This can actually be an incidental finding as the patient is relatively healthy other than the lymphocytosis, i.e. it may be diagnosed during routine blood work in an older patient.
- The lymphocytosis will progress over time although initially, if more mild, may be difficult to differentiate from a reactive lymphocytosis on routine blood work alone.

- It was termed ‘chronic’ as human patients tended to live longer with this type of leukemia (vs. the acute form).

SUMMARY OF FLOW FOR SMALL CELL NEOPLASTIC POPULATIONS


DOG: Immunophenotyping by flow cytometry
Mixed population of B and T cells (heterogeneous)
Reactive lymphocytosis
Phenotypic homogeneity
CD21 + (B cells)
Larger cells
More aggressive
Smaller cells
Less aggressive
CD8+ cells (T cells)
E. canis positive
Monitor response to TX
E. canis negative
Intially <30,000 lymphocytes: better prognosis
Intially >30,000 lymphocytes: more aggressive
CD4+ cells (T cells)
CD45 +, Low MHC II
More aggressive disease course
CD45-, High MHCII
Less aggressive disease course (indolent)
BULL DOGS
Low CD25 (B cell)
Good prognosis
CD4-/ CD8- (T cell)
Poor prognosis



APPROACH TO LARGE CELL/IMMATURE LYMPHOCYTOSIS

When immature cells are identified, cells may be distinctly lymphoid but it is important to know that morphology can be an insensitive indicator of lineage (i.e. morphology may not be able to distinguish between myeloid and lymphoid for immature cells). If the cell morphology is thought to be lymphoid, it is either due to stage V lymphoma or lymphoid leukemia (ALL). Smear review alone cannot distinguish between stage V lymphoma or ALL. However, because prognosis can be quite different between ALL and stage V lymphoma, additional testing can be pursued to help distinguish the two. Acute lymphoid leukemias often have CD34+ on flow cytometry, are more likely to be associated with other cytopenias and lack of tissue involvement (i.e. less likely to have node enlargement or organomegaly).

5. CBC findings that support an immature lymphocytosis due to lymphoid neoplasia

- Acute lymphoid/lymphoblastic leukemia (ALL) – An increase (mild to marked) in immature lymphoid cells which has originated in the bone marrow
  - These cells are blasts and cannot be easily differentiated from other blasts, i.e. myeloblasts, undifferentiated blasts, so this may be termed 'acute leukemia' when morphology is difficult to differentiate.
- Stage 5 lymphoma
  - Lymphoblasts/large lymphoid cells are seen in peripheral blood – this is uncommon (compared to the typical presentation of lymphoma).
  - Need additional info to differentiate this from ALL (e.g. flow cytometry)

Chemistry findings (may or may not be present):

- ± Hyperglobulinemia in some types of B cell lymphoid neoplasms
- ± Hypercalcemia in some types of lymphoma