Different approaches for the diagnosis and classification of canine Lymphoma

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Overview

• Background
• Classification
  • Development of classification systems
  • WHO classification for dogs
• Diagnosis
  • Cytopathology
  • Histopathology
  • Immunotyping
    • Immunohistochemistry
    • Flow cytometry
  • Clonality testing
• Importance of classification
  • Current literature
  • Future directions
Canine Lymphoma - Background

- Most common hematopoietic tumour of humans and dogs
- Similar to human non-Hodgkin’s lymphoma
- Immunoblastic B cell lymphoma most common type
- T cell lymphoma usually associated with poor outcome
- Typically diagnosed by cytopathology
- Treatment: combination chemotherapy
Timeline of Lymphoma Classification - Humans

Rappaport

Lukes and Collins

Kiel

Working Formulation

Revised European-American Lymphoma (REAL)

World Health Organization (WHO)

Nodular or diffuse + cytomorphology

Cytomorphology + immunotype (B or T) + survival data

Histopathology + cytomorphology, + immunotype (multiple markers) + genetics + clinical data

Nodal and extranodal
WHO Classification of Lymphoma

Humans: >100 recognized subtypes
Dogs: 30 recognized subtypes, but only a few are common

WHO Classification - Dogs

- First standardization

- WHO classification
  - 20 pathologists: 83% consensus based on histopathology
  - Reproducibility by pathologist: 40% to 86.7% (mean 65.5%)

- Limitations of WHO classification in dogs
  - Some entities unique to dogs
  - Some entities differ between dogs and humans
  - Limited genetic information of different subtypes
  - Lack of prognostic information for most subtypes
Cytopathology

• Quick, minimally invasive, inexpensive and reliable
  • Up to 90% sensitivity/specificity.

• Cannot assess tissue architecture or immunotype

• More suitable for diffuse than follicular tumours

• Difficult to access extranodal sites
Histopathology

- More invasive and expensive than cytopathology
- Both cells and architecture are assessed
  - Cytomorphology
  - Diffuse vs follicular
  - Capsule thinning / invasion
  - Tingible-body macrophages
  - High endothelial venules
  - Mitotic index
Immunohistochemistry (IHC)

- Formalin fixed or frozen tissue
  - Epitope alteration with formalin fixation (i.e. CD4, CD8)

- Can readily determine B or T cell
  - T cell: CD3
  - B cell: CD20, Pax-5, CD79a*

- Expense in addition to histopathology
Flow Cytometry (FC) - Dogs

- Lymph node aspirates (22g) into saline
- Multiple markers on a large number of cells assessed concurrently and quantitatively
- Relatively new technique with limited availability and standardization to date
- Requires specialized equipment and expertise
- Use to type but not to diagnose lymphoma
- Cost variable
Clonality Testing

- PCR for antigen receptor rearrangement (PARR)
  - Specific primers for amplifying lymphocyte antigen receptor genes - clonal or polyclonal product

- Any sample with enough tumour cell DNA, including formalin fixed tissue
  - Interpretation in conjunction with histopathology and IHC

- Not a silver bullet for diagnosing lymphoma
  - Reserved for cases where cytopathology and histopathology insufficient (i.e. early marginal zone lymphoma, follicular lymphoma)

- Not used for immunotyping
  - Cross-lineage rearrangement
<table>
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<th>IHC</th>
<th>FC</th>
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Testing algorithm for suspected lymphoid neoplasms

Morphology
- Cytology
  - Lymphoma or leukemia
  - Equivocal
- Histopath
  - Equivocal
  - Lymphoma

Immunophenotype
- Flow cytometry to classify
  - CD3/4/5/8 T or CD21/22 B cell lymphoma
- IHC
  - Equivocal
- IHC
  - T or B cell lymphoma

Clonality
- Equivocal
- Clonality
  - Lymphoma

Adapted from: AHL Labnote 44, 2016
Why is WHO classification important?

- In human medicine each lymphoma subtype has a unique outcome
  - Information is updated frequently

- Limited prevalence and prognostic data

- Prognostication allows informed decision making
  - Subtypes may respond differently to therapy
Valli et al 2013

• Large scale survival analysis based on histopathology classification (992 dogs with lymphoma)

• DLBCL is most common (~66% of cases)

• T zone lymphoma (TZL): Long survival without therapy

• Several limitations:
  • No flow cytometric data hence only T or B immunotype
  • Small sample size for rare types
  • Non-standardized therapy
Avery et al 2014

- Survival analysis of dogs with CD3+, CD4+, CD45+, and MHC class II- lymphoma based on flow cytometry.

- Median survival time 159 days

- Worse outcome if CD5+

- Limitations
  - No histopathology
  - Combined peripheral T cell lymphoma - not otherwise specified (PTCL-NOS) and T lymphoblastic lymphoma (T-LBL)
Seelig et al 2014

- Histopathology and flow cytometry on CD3⁺, CD45⁻, MHC class II⁺, and CD21⁺ lymphoma
- Phenotype consistent with T zone lymphoma
- Indolent course of disease
- Primarily CD4⁻/CD8⁺, but also CD4⁺/CD8⁻ and CD4⁻/CD8⁻
Flow cytometric data / survival analysis on 127 dogs with T cell lymphoma (TCL)

Summary:

• Hepatosplenic and gastrointestinal T cell lymphoma had worse outcome than multicentric TCL

• Expression of MHC class II = better outcome

• CD4⁻/CD8⁺/MHCII⁻ immunotype = more indolent disease

• Concurrent leukemia: shorter survival than without leukemia

*Under review: Vet Immunol Immunopathol, 2017*
Kaplan-Meier plots of survival (A) and progression free interval (B) in relation to immunotype
Our Research: Prospective Study

Multimodal diagnostic approach to classification of TCL

- Cytopathology
- Flow cytometry
- Histopathology
- Immunohistochemistry
  - Formalin fixed sections (ffIHC)
  - Frozen sections (fsIHC)
Results

• Expression of CD3 on cell membrane (FC) vs in cytoplasm (ffIHC) is variable

• Immunotype of TZL is variable in FC vs fsIHC

• CD8+ T cell lymphoma has low expression of proliferation marker Ki67
Conclusions and Future Directions

• Refined diagnosis, classification and prognosis of lymphoma is possible, and may be warranted in dogs to be treated with chemotherapy (~$5,000)

• Different histopathological and immunotypic subtypes of lymphoma are associated with different outcomes

• Standardization of current testing could be improved

• The current body of evidence should be expanded with large prospective multi-center studies