Lymphoma: a heterogeneous disease

Application of the WHO Lymphoma Classification Scheme

Peter F. Moore
WHO - classification

- Extension of REAL classification - 1994 ILSG
- Broadened to include myeloid, mast cell and histiocytic neoplasia
- Disease entities defined:
  - Lineage and postulated cell of origin
  - Morphology and Immunophenotype
  - Genetic features and Clinical features
WHO - classification

- Lymphoid neoplasia: B cell, T cell, NK cell and Hodgkins lymphoma

- Lymphomas and leukemias considered together - may be manifestations of the same tumor
  - B-CLL and B cell small lymphocytic lymphoma
  - Lymphoblastic lymphoma and lymphoblastic leukemia
WHO - classification

- **B and T/NK lymphomas:**
  - Precursor cell lymphomas
  - Mature cell lymphomas

- **Non-Hodgkin lymphomas -**
  - Distinct diseases
  - Distinctive clinical features/epidemiology
  - Distinctive responses the therapy.
WHO classification of tumors of hematopoietic and lymphoid tissues

- Proponents of other schemes have endorsed WHO classification
- First true world-wide consensus classification scheme for hematologic malignancies
- ACVP initiative - Lymphoma Study Group - investigated suitability of WHO scheme for animal lymphomas (led by Dr. Ted Valli)
WHO lymphoma classification - Precursor B/T

B/T

- B acute lymphoblastic leukemia (B-ALL)
- B lymphoblastic lymphoma (B-LBL)
- T Acute lymphoblastic leukemia (T-ALL)
- T lymphoblastic lymphoma (T-LBL)
WHO Lymphoma classification - Mature B cell

- Diffuse large BCL
- Follicular BCL
- Mantle cell BCL
- B-CLL/Small lymphocytic BCL
- Burkitt-like BCL
- Extramedullary Plasmacytoma
- Multiple myeloma
- Marginal zone BCL
- Nodal Splenic MALT
- Centroblastic
- Immunoblastic
- T cell/histiocyte rich
- Anaplastic
- Indolent - initially
WHO Lymphoma classification - Mature T cell

Nodal TCL
- Peripheral TCL - unspecified
- T-zone TCL
- Anaplastic large TCL
- Angioimmunoblastic TCL

Cutaneous TCL
- Mycosis fungoides
- Pagetoid reticulosis
- Sézary syndrome
- Peripheral TCL - unspecified

Extranodal - other
- Enteropathy associated TCL
- Hepatosplenic TCL
- Peripheral TCL - unspecified

LGL leukemia
- T-LGL CLL
- T-LGL ALL

LGL lymphoma

Indolent - initially
Indolent - some forms
Integrative diagnostics - Leukocytic diseases

- Clinical and clinico-pathological data
- Morphological data - histology/cytology
- Immunophenotyping - reagent panels
- Molecular assessments - antigen receptor clonality for lymphoma
Lymphocyte Development
and
Antigen receptor gene rearrangement
T cell receptor gene rearrangement

- **TCRG** - molecular clonality target (rearranged in γδ and αβ T cells)

![Diagram of T cell receptor gene rearrangement]

- TdT n base addition
- Variable
- Joining
- Constant
- TCRγδ
- TCRαβ
- TCRG/TCRD
- TCRA/TCRB
- CD3
B cell receptor gene rearrangement

- Ig heavy chain (IGH) locus - molecular clonality target
- V segment mutation in germinal center B cells - reduces sensitivity

Variable

Diversity

Joining

Constant

TdT n base addition

IgM

CD79
Feline TCRG V-N-J alignment CDR3 region

5' primer

3' primer

K L Q K S D E G V Y Y C A A W E A S
K L Q K S D E G V Y Y C A A W E A S
K L Q K S D D V Y Y C A A W E P
K L Q K S D E G V Y Y C A A W E R G T
K L Q K S D E G V Y Y C A A W E A V D
K L Q K S D E G V Y Y C A A W E A R G T
K L Q K S D E G V Y Y C A A W E A C G T
K L Q K S D E G V Y Y C A A W E A P A K
K L Q K S D E G V Y Y C A A W E A A T G G
K L Q K S D E G V Y Y C A A W E A R
K L Q K S D E G V Y Y C A A W E A X T
K L Q K S D E G V Y Y C A A W E A R G T
K L Q K S D E G V Y Y C A A W E A R D N L G
K L E K S D E G V Y Y C A A W E A R
K L A K S D E G V Y Y C A V W E D
K L A K S D E G V Y Y C A V W E D P A D
K L A K S D E G V Y Y C A V W E V G

3' V segment
CDR3
J segment

variability
Ag receptor gene rearrangement - *indications*

- Morphological, cytological, immunophenotypic properties inconclusive
  - Lack of architectural effacement in organized lymphoid tissue - MZL or TZL
  - Lamina proprial or intra-epithelial lymphocytosis in the small intestine
  - Lympho-histiocytic proliferations in skin
Canine “inflamed” T cell lymphoma (PTCL)

- 387403 - Bernese Mtn dog, MC, 6 yrs - masses on digit, carpus, mandible.
- **Dec 07** - DX#1: Histiocytic dermatitis
- **Mar 08** - DX#2: Histiocytic sarcoma
- **May 08** - DX#3: Reactive histiocytosis
387403 - Bernese mountain dog, MC, 6 years
DX: Non-epitheliotropic T cell lymphoma (& lympho-histiocytic dermatitis)
Molecular clonality - **limitations**

- Sensitivity limited with high polyclonal background
  - Miss small clonal populations - e.g. inflamed lymphoma
- Sensitivity limited - B cell lymphoma - *IGH* V mutation
- Clonality is not equivalent to malignancy
  - Interpret results in appropriate context
- *IGH* and *TCRG* rearrangements are lineage associated - but not absolute markers of lineage
  - Cross lineage rearrangements in lymphoid and myeloid malignancies
WHO lymphoma classification - Precursor B/T

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- B acute lymphoblastic leukemia (B-ALL)
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- T lymphoblastic lymphoma (T-LBL)
T-lymphoblastic lymphoma (T-LBL)

- Mass lesion: T-LBL
  - mediastinum, LNs, spleen, other sites
- Predominance of blood/BM involvement: T-ALL
T-lymphoblastic lymphoma

- **Origin**: Precursor T lymphoblast
- Hypercalcemia a common feature
- High grade rapidly progressive
- Loss Cfa 11 in high-grade TCL
- P16 (Rb) deletion/inactivation in all cases
WHO Lymphoma classification - Mature B cell

Diffuse large BCL

Centroblastic

Immunoblastic

T cell/histiocyte rich

Anaplastic

Marginal zone BCL

Nodal

Splenic

MALT

Follicular BCL

Mantle cell BCL

B-CLL/Small lymphocytic BCL

Burkitt-like BCL

Extramedullary Plasmacytoma

Multiple myeloma

Indolent - initially
Germinal Center Responses

- Mantle zone
- Light zone: Ig affinity maturation, Ig class switch
- Dark zone: IGH-V mutation
Marginal Zone

Mantle zone

GC - Light zone

GC - Dark zone

MZL

MCL

FL

DLBCL

MZH
Diffuse Large B cell Lymphoma (DLBCL)

- Centroblastic
- Immunoblastic
- T cell/histiocyte rich
- Anaplastic
Diffuse Large B cell Lymphoma

- **Origin:** centroblasts in GC dark zone
- Lymph nodes; spleen; extranodal
- Most prevalent lymphoma in dogs
- High grade lymphoma - high proliferative fraction
Spleen - diffuse large B cell lymphoma

Canine spleen - splenomegaly due to white pulp infiltration/obliteration
DLBCL - Centroblastic
DLBCL - Immunoblastic
00B1609 - QH mare, 11yrs - Skin masses 2yrs

amyloid

DLBCL - T cell rich
T cell rich B cell lymphoma - equine skin
DLBCL - Anaplastic

CD20
Marginal zone lymphoma (MZL)

- Nodal - most common
- Spleen - solitary mass and/or diffuse
- Extranodal - MALT lymphoma - rare
Marginal zone lymphoma

- **Origin**: LN - perifollicular MZ B cells (chronic follicular hyperplasia) (dogs)
  
  Splenic MZ B cells (dogs)
  
  BALT and NALT - cats - rare

- **DX**: architecture + cytologic characteristics

- **DDX**: nodular hyperplasia when spleen involved

- Indolent lymphoma - low proliferative fraction

- May evolve into DLBCL
Spleen - marginal zone lymphoma

MZL- solitary mass and diffuse involvement. Perifollicular marginal zones slowly coalesce.
Marginal zone hyperplasia - lymph node
Follicular lymphoma (FL)

- Nodal - most common
- Splenic
- Extranodal
Follicular lymphoma

- **Origin**: Centrocytes in GC light zone
- **DX**: architecture + cytologic characteristics
- Indolent B cell lymphoma - low proliferative fraction
- May evolve into DLBCL
- **Human**: t(14:18) - BCL2 gene rearranged
Mantle cell lymphoma (MCL)

- Nodal
- Spleen - 3 dogs (clonal IGH)
- Bone marrow
- Extranodal - GI tract
Mantle cell lymphoma

- **Origin:** B cell from inner mantle zone
- **DX:** architecture + cytologic characteristics
- Solitary nodular mass in the spleen of dogs
- **DDX:** splenic nodular hyperplasia in dogs
- **Indolent B cell lymphoma - low proliferative fraction - dogs; more aggressive in humans - esp. blastoid variant**
- **Human:** CD5+, BCL2+, Cyclin D1+
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Extranodal - other
- Enteropathy associated TCL
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LGL leukemia
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- T-LGL CLL
- T-LGL ALL

T-zone TCL

Peripheral TCL - unspecified
Peripheral T cell lymphoma - PTCL

- Heterogeneous group
- Nodal
- Skin (non-epitheliotropic TCL)
- Generalized (2° leukemia common)
Peripheral T cell lymphoma - PTCL

- **Origin:** Peripheral T cells
- High-grade lymphoma - high proliferative fraction
- Cytology extremely variable
- Inflamed lymphoma - esp. cutaneous PTCL
- DDX: Reactive (cutaneous) histiocytosis
- P16 (Rb) deletion/inactivation in all cases
T-zone lymphoma (TZL)

- Nodal
- Human - variant of PTCL - i.e. high-grade lymphoma
T-zone lymphoma

- **Origin:** Peripheral T cells
- Variable LN involvement (1, 2 or generalized)
- Indolent lymphoma (dogs) - years
- Low proliferative fraction - mitotic rate low - (if not - PTCL)
- 2º leukemia observed - prognosis unaffected
- DDX: paracortical hyperplasia (TCRG clonality) 
  Marginal zone BCL (MZL) - requires IHC
Hepatosplenic lymphoma (HS-TCL)

- Spleen
- Liver
- Bone marrow
- Generalized lymphadenopathy lacking
Hepatosplenic T cell lymphoma

- **Origin**: splenic red pulp $\gamma\delta$ T cell

- Cytology - LGL. Usually TCR$\gamma\delta$+ CD11d+

- $2^\circ$ hemophagocytic syndrome common (CD11d+ macrophages activated); malignant T cells erythrophagocytic

- Clinical - aggressive course, anemia, thrombocytopenia (immune mediated??)

- DDX: hemophagocytic histiocytic sarcoma
Splenic red pulp - CD11d+ diseases

- CD11d
- WP
- MZ
- T-CLL - LGL type
- Hemophagocytic HS
- Hepatosplenic lymphoma

RP
Lymphocyte Trafficking
and
Tissue Localization of Disease
Lymphomas of skin and gut
T cell lymphomas of skin and gut

Marked species differences

incidence

behavior

immunophenotype
αβ T cells

- Naïve T cells - exported from the thymus
  - Recirculate between blood and lymph nodes

- Effector memory T cells - wide migratory range
  - Recirculate between blood and cutaneous or mucosal sites

- Central memory T cells - retain migratory path of naïve T cells
Lymphocyte recruitment - to skin

How are the migratory pathways of naïve lymphocytes redirected to skin?

Dendritic Cell Imprinting
Naïve T cell

LN Paracortex

Dendritic Cell

Home to SKIN

Dermal endothelium

E-selectin

CCL17

CLLA

Fucosyl Tr VII

αLβ2

Memory T cell
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LGL lymphoma

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Cutaneous Lymphoma

- Epitheliotropic TCL
  - Mycosis fungoides
  - Pagetoid reticulosis
  - Sézary syndrome
- Non-epitheliotropic PTCL
- Non-T non-B lymphoma
- B cell lymphoma (Diffuse large BCL)
- Plasmacytoma
Skin homing T cell lymphoma
Epitheliotropic T cell lymphoma - skin

- Mycosis fungoides (MF) - lesions confined to skin for extended period - clinical course up to 4 yrs.
- MF is a disease of skin homing memory T cells
- Dissemination initially occurs within the skin and skin draining lymph nodes
- Evidence of dissemination - identical T cell clone found in multiple skin sites
CD3

Epidermis

Hair follicle
Canine Mycosis Fungoides

Immunophenotype

- Consistent expression of CD3 (n = 56)
- CD8+ (80% cases) or CD4-CD8- (20% cases)
- Memory cell phenotype (CD45+CD45RA-CD49d+)
- Marked contrast to human MF - TCRαβ+CD4+
Canine Mycosis Fungoides

- T CELL RECEPTOR USAGE?
- Development program for TCR specific probes
- Mab specific for TCR$\alpha\beta$ and TCR$\gamma\delta$ developed
Canine MF - TCR Expression

- TCR immunophenotype in MF all forms
  - TCR$\alpha\beta+$: 21 cases (40%)
  - TCR$\gamma\delta+$: 32 cases (60%)
- Canine MF involves $\gamma\delta$ T cells at much higher incidence than human MF
Canine MF - TCR Expression

**Classical MF:** $\text{TCR}^{\alpha\beta+} \approx \text{TCR}^{\gamma\delta+}$ \hspace{1cm} (n=38)

**Pagetoid MF:** $\text{TCR}^{\gamma\delta+}$ \hspace{1cm} (n=15)
Pagetoid MF: a lymphoma of γδ T cells
Canine MF - pagetoid reticulosis

Exclusive expression of TCR\(\gamma\delta\)

Clonal origin from resident epidermal \(\gamma\delta\) T cells

Prolonged expansion entirely within the epidermis
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  - T-LGL ALL
- LGL lymphoma
  - Indolent - initially
  - Indolent - some forms
Gastrointestinal lymphoma

- Enteropathy associated TCL (EATCL)
  - small cell
  - large cell
  - LGL
- Diffuse large BCL
Enteropathy associated TCL

- **Origin**: intestinal homing T cell (IEL or LPL)
- Small intestine - high prevalence in cats
- IBD: precursor lesion in most cats - distinction (TCRG clonality)
- Small cell - indolent
- Large cell (LGL) - aggressive high grade
- Architecture - *mucosal* or *transmural*
Mucosal homing T cell lymphoma
Feline small intestine: diffuse MALT

- IELs - distinctive phenotypic subsets versus PBL
- Expression of β7 integrins (α4β7) linked to mucosal homing
- Feline IEL (30%) granulated - perforin, granzymes
- CD8αα T cells predominate - role in immune surveillance
- Feline IEL (70%+) express the mucosal integrin - CD103 (αEβ7)
04B0314 - Feline, DLH, FS, 13 yrs

Mucosal epitheliotropic T cell lymphoma - duodenum - endoscopic
**TCRG clonality - 04B0314 -Feline, DLH, FS, 13 yrs**

Endoscopic biopsy - duodenum and stomach
Mucosal lymphoma - cytology

- Small lymph: 68
- S-GL: 12
- L-GL: 3
- Large lymph: 2

Total: 85
Mucosal lymphoma - survival

Dead  
13.9 ± 13.1m (0.5 - 46m, n=29)

Alive  
24.2 ± 17.1m (4 - 51m, n=16)
Jejunal mass: transmural LGL T cell lymphoma
Transmural T cell lymphoma - survival

Dead

9 L-GL
2 L-lymph

2.9 ± 4.8m (0.1 - 15m)

Alive

1 S-lymph
1 L-GL

28m
2m
Lymphoma

- WHO classification scheme is applicable to canine (feline) lymphoma
- Basic immunophenotyping often needed (B/T)
- Molecular clonality necessary in some instances (TZL, MZL, MCL, FL and T cell/histiocyte rich BCL)
- Recognition of homogeneous lymphoma groups will lead to tailored therapies and discovery of underlying molecular defects
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