

Neoplasia

Advanced diagnostics

Dr Maurice Zandvliet, DVM, MVR, PhD, Dipl ECVIM-CA (Med & Onc)
UU Animal Cancer Centre - Medical and Radiation Oncology

1

How to diagnose neoplasia?

- Signalment
- Physical examination
- Diagnostic imaging
- Routine clinical pathology (hematology/clinical chemistry)
- Advanced clinical pathology
 - Hormones, Proteins, Biomarkers
- Cytology
- Histopathology
- Immunocytochemistry/Immunohistochemistry
- Flow cytometry
- DNA-tests
- Cytogenetics

2

Abby

bobtail F 3½Y



Not doing well for 2 weeks: eating less, more "tired", last heat 4 months ago

Physical examination: unremarkable

- No improvement with NSAID's

After 2 weeks

- Clinical pathology:
 - Mild anemia (38%), mild thrombocytopenia (102 G/L)
 - Mild increase in ALP (108 IU/L)
- Diagnostic imaging:
 - Thoracic radiographs unremarkable
 - Abdominal US: mild hepato-splenomegaly, no pyometra
- Serology
 - Negative for *Anaplasma*

3

Abby

bobtail F 3½Y



Now owners get worried, they lost their other dog to cancer
"Question doc, could this be cancer??"

Referring veterinarian:

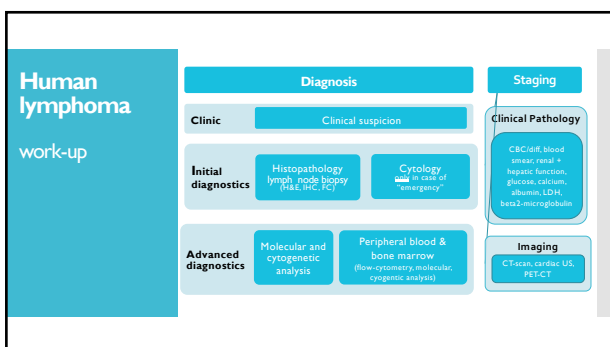
- Repeat PE: mild generalized lymphadenopathy.....
- Referred Abby to oncology service: Does Abby have lymphoma?

Oncology service

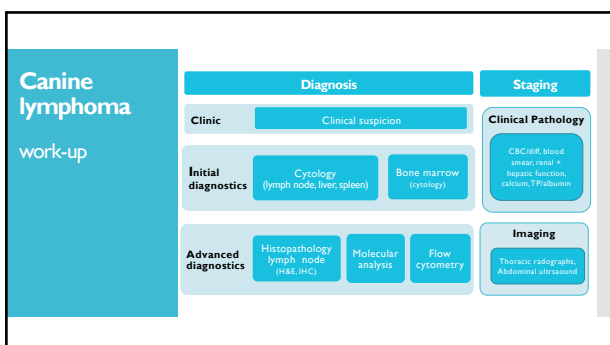
- P.E.: mild generalized lymphadenopathy, otherwise unremarkable
- Cytology:
 - monomorphic population of intermediate-sized lymphoid cells
 - absence of reactive lymphoid component.

Atypical lymphoid proliferation, suspect lymphoma.

4



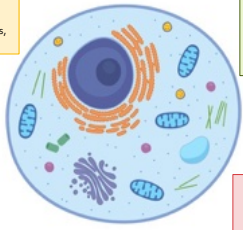
5



6

What can we look for?

DNA
= Mutations, PARR



Cells (morphology)
= Cytology or Histology

Proteins
= Immunochemistry, Flow-cytometry

7

What are we looking for?




8

Canine lymphoma biomarkers

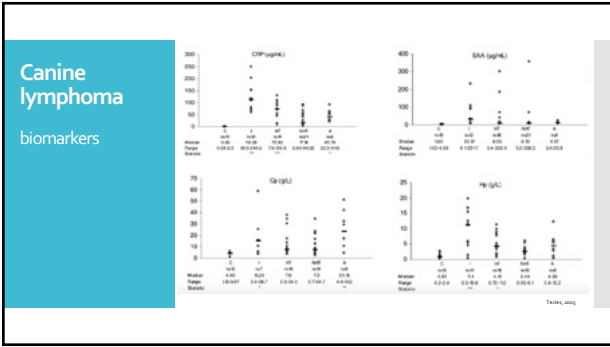
Various targets have been evaluated:

- calcium
- LDH iso-enzymes
- acute phase proteins
 - alpha-1-acid glycoprotein
 - C-reactive protein
 - haptoglobin
- Nucleosomes
- glutathione-S-transferase (ROS)
- VEGF/MMPs
- serum Thymidine Kinase 1 (TK-1)
- circulating cell-free DNA
- microRNA expression patterns

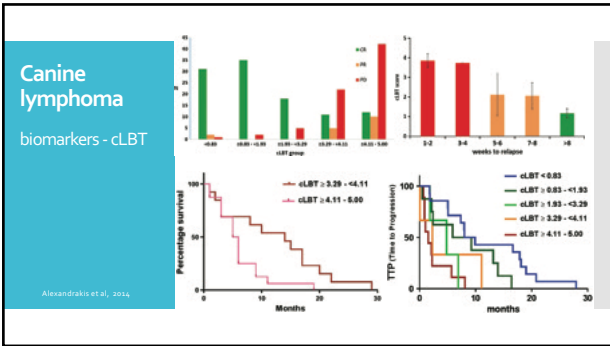
9



10



11



12

13

14

15

[illegible][illegible][illegible]

Canine lymphoma

cytology

Martini 2021

Received 21 March 2021 | Accepted 10 November 2021

DOI: 10.1111/jvim.15488

STANDARD ARTICLE

Journal of Veterinary Internal Medicine

ACVIM

Performance of lymph node cytopathology in diagnosis and characterization of lymphoma in dogs

	Sensitivity (%)	Specificity (%)
Lymphoma	92.6 (88.8-95.1)	89.4 (79.0-95.0)
High grade B cell lymphoma	68.8 (62.7-74.3)	60.9 (51.2-69.8)
Low grade B cell lymphoma	17.9 (12.3-25.4)	90.1 (86.6-92.7)
High grade T cell lymphoma	72.9 (57.4-84.3)	82.5 (77.6-86.4)
Low grade T cell lymphoma	82.1 (59.1-93.6)	95.4 (93.2-97.0)

19

Immunochemistry

1 Sample collection

2 Immunohistochemistry assay

3 Microscopy and data analysis

https://www.biomed.com/meda/image/02a/8d/Principle_IHC_EN.png

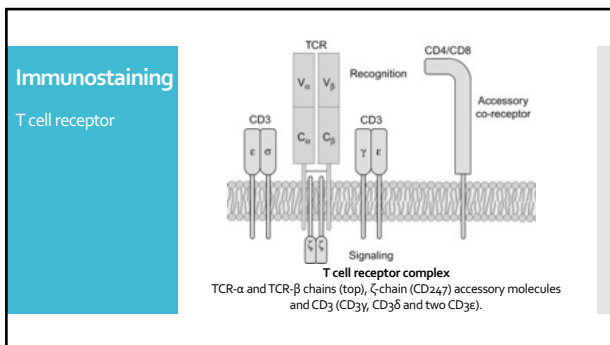
20

Immunostaining

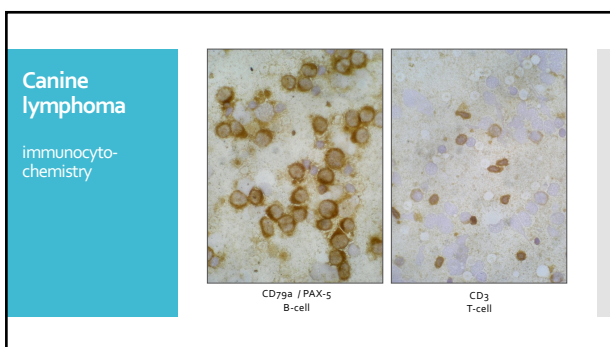
B cell receptor

B cell receptor (BCR)
transmembrane protein on the surface of B cells consisting of a membrane-bound immunoglobulin molecule and a signal transduction region (CD79). Disulfide bridges connect the Ig-isotype and the signal transduction region.

21



22



23

Canine lymphoma

histology - classification

Summary of Canine Malignant Lymphoma Revised From the Revised European-American Classification of Lymphoid Neoplasms/WHO Classification of Lymphoid Neoplasms	
B Cell Neoplasms Precursor B cell neoplasms Precursor B lymphoblastic leukemia/lymphoma Plasma (plasmablastic) B cell neoplasms B cell chronic lymphocytic leukemia/prolymphocytoma Leukemic/lymphomatous lymphoma B cell prolymphocytic leukemia Lymphoplasmacytic lymphoma Splenic marginal zone B cell lymphoma Plasma cell myelomatous neoplasms Extramedullary marginal zone B cell lymphoma of mucosa-associated lymphoid tissue type Nasal marginal zone lymphoma Follicular lymphoma Mantle cell lymphoma Diffuse large B cell lymphoma ¹ Multicentric large B cell lymphoma Burkitt's lymphoma/Burkitt's cell leukemia Brevicentric/enteric high-grade B cell lymphoma B-cell histiocytoma ² Primary effusion lymphoma	T Cell and Plasmacytic Natural Killer Cell Neoplasms Precursor T cell neoplasms Precursor T lymphoblastic leukemia/lymphoma Plasma (plasmablastic) T cell and natural killer cell neoplasms T cell prolymphocytic leukemia Large granular lymphocytic leukemia (LGL) Aggressive natural killer (NK) cell leukemia Peripheral T cell lymphoma, unspecified ³ Adult T cell lymphoma/leukemia Invariant T cell lymphoma (T-lymphoma associated) Histiocytic T cell lymphoma Subcutaneous panniculitis-like T cell lymphoma Mycosis fungoides/Sezary syndrome Angioimmunoblastic T cell lymphoma, T and null cell primary cutaneous type Peripheral T cell lymphoma, not otherwise specified Angioimmunoblastic T cell lymphoma Angioimmunoblastic T cell lymphoma

¹ Peripheral T cell lymphomas are those that are not otherwise specified (NOT) in a specific subtype by further definition. See FIGS. 2(a) & 4.

25

Canine lymphoma

Histology - classification

Histological classification schemes

- 1940: Gall and Mallory
- 1966: Rappaport
- 1976: Lukes-Collins
- 1978: Kiel
- 1982: Working Formulation
- 1988: Updated Kiel
- 1994: REAL
- 2000's: WHO

Seeliger et al., 2016

1. Architecture

2. Cell Size

3. Phenotype

27

Immunohistochemistry

round cell tumor panel

Marker	PAX-5 / CD79a	CD3	Melan A	kit	CD18	Iba-1	MUM1	Kappa / Lambda
Neoplasia								
Histiocytic					+	+		
Lymphoid	B-cell	T-cell			+		±	
Plasma cell	+				+		+	+
Mast cell				+				
Amelanotic melanoma			+					

Other commonly used diagnostic IHC-markers:
Cytokeratin (epithelial), Vimentin (mesenchymal), Desmin (myogenic), vWF/FactorVIII (endothelial), Hormones (PTH, ACTH, Thyroglobulin, Calcitonin, Chromogranin/Synaptophysin, Insulin)

28

Canine lymphoma

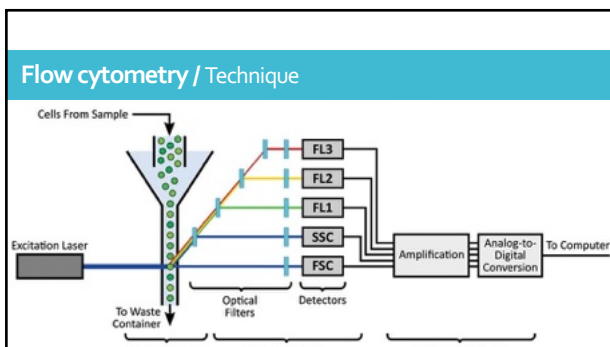
cDLBL

Valli et al., 2011

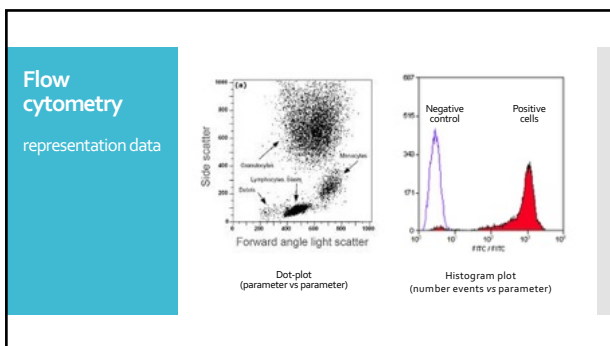
29



32



33



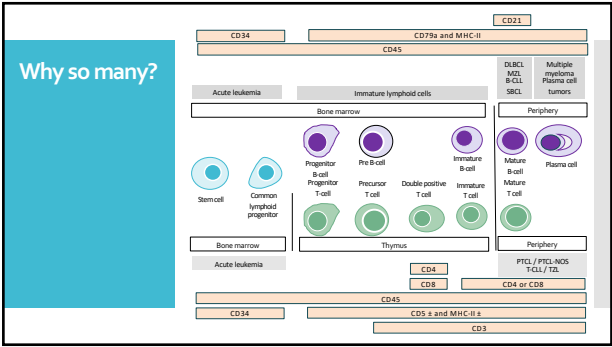
34

Canine lymphoma

Flow cytometry

Marker	Cells
CD1	Dendritic cells
CD3	Pan-T cells
CD4	Helper T-cells; neutrophils
CD5	Pan-T cells
CD8	Cytotoxic T cells
CD14	Monocytes
CD18	Pan-leukocytes
CD19/20/21/22	Mature B-cells
CD34	Hematopoietic stem cells
CD45	Non-erythroid hematopoietic cells
CD79a/β	Pan-B cells (incl. some plasma cells)
MHCII	Antigen-presenting cells, most lymphocyte types
Surface IgM	Immature B cells
Light-chain	Antigen-producing cells, plasma cells
CD138	Plasma cells

42



43

Flow cytometry

Results

- Differentiate cells on cell size and "internal complexity" (FSC-SSC)
- Staining with specific antibodies to detect specific epitopes ("CD")
- Useful for diagnosing and characterizing lymphoproliferative diseases including lymphomas and leukemias
 - useful for subtyping some histological T cell, but not B cell diseases

Diagnostic criteria

- Over-representation of a single population
- Aberrant expression

44

Enlarged lymph nodes

Lab. retriever MN 7Y

45

Enlarged lymph nodes

Lab. retriever MN 7Y

B cell lymphoma

46

Enlarged lymph nodes

mixed breed MN 8Y

T zone lymphoma

48

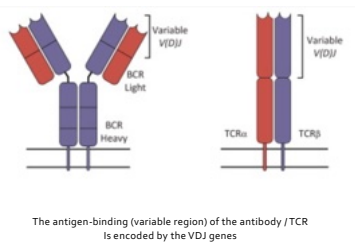
DNA-based tests

1. Identify a specific mutation
 - Diagnostic
 - BRAF – urothelial carcinoma
 - RCND – renal cell carcinoma (German shepherd)
 - Therapeutic
 - c-Kit- mutation – mast cell tumor
 - MDR1-Δ – deletion mutation, increased drug sensitivity
 - Diagnostic, prognostic and therapeutic
 - Searchlight DNA – next generation sequencing 120 cancer genes
2. Demonstrate clonality
 - Diagnostic / Staging
 - PARR – lymphocyte clonality
 - MRD (minimal residual disease) – lymphoma

53

Canine lymphoma

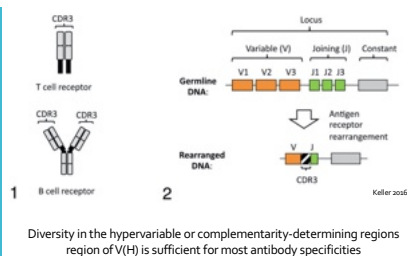
PARR



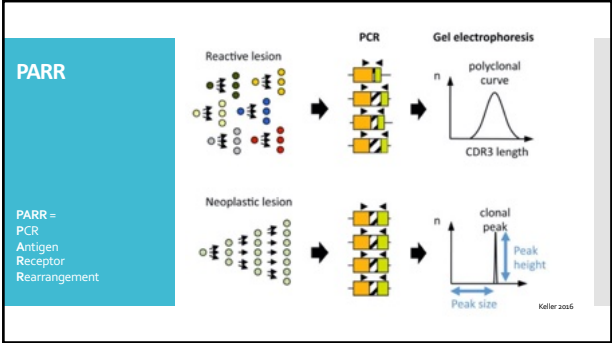
54

PARR

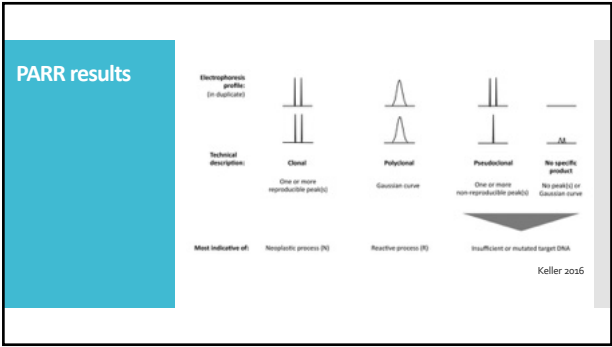
Ig/TCR genes are assembled through random assortment of V (86), D (6) and J (6) genes and insertion of a random number of nucleotides



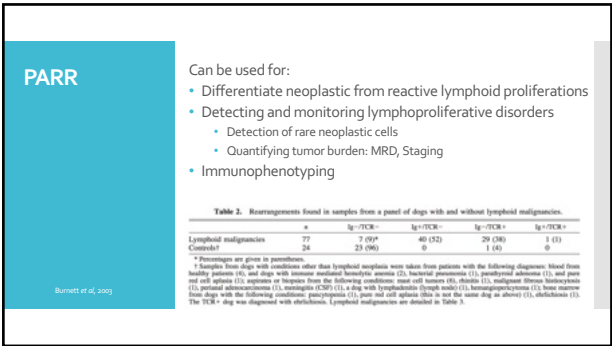
55



56



57



59

Abby

bobtail F 3½Y



So what's next??

- Nu.O®-test: Caution zone
- Flow-cytometry peripheral lymph node:
 - Higher % T cells, abnormal ratio CD4+/CD8+
 - No aberrant expression
 - High Ki-67 expression
- PARR: monoclonal T cell and polyclonal B cell proliferation
- MDR1-ΔΔ: N / P

Conclusion:

- high grade T-cell lymphoma (PTCL-NOS)
- carrier of MDR-1 deletion
