Advances in the diagnosis and treatment of canine lymphoma

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Outline

• Diagnosis
  – Immunophenotyping
    • PARR
    • Flow Cytometry
  – Case studies

• Treatment
  – Monoclonal antibodies
  – Other new therapeutics under investigation
Diagnosis of Lymphoma

• Cytology or Histopathology
  – Cytology is usually adequate for the diagnosis of lymphoma
  – Histopathology provides additional information
    • Tissue architecture
    • Cell distribution
    • Mitotic figures
    • Certain nuclear features

• Flow Cytometry
• PARR
Immunophenotyping

Can be used in several situations

• To confirm the diagnosis
• To get more information regarding cell characteristics (prognosis)
• Molecular remission
B cell Makers

- B cell
  - Cell surface
    CD20, CD21, CD22
  - Cytoplasmic/nuclear
    CD79a, Pax5, CD20c, Mum1/IRF

*Flow Cytometry, IHC and permeabilization
T cell Makers

• T cell
  – Cell surface
    CD3, CD4, CD5, CD8
    *Flow Cytometry
  – Cytoplasmic
    CD3c
    *Flow Cytometry, IHC and permeabilization
Flow cytometry

• Detects the expression of cell surface and intracellular antigens, as well as their level of expression

• Antibodies against different cell surface or intracellular proteins are conjugated to different fluorescent antibodies

• The degree of light is proportional to the amount of antibody bound
Flow cytometry
Flow cytometry - results

Canine and Feline Cytology 2nd Edition
Flow Cytometry – sample submission

• Sample preparation:
  – Blood: EDTA tube, >0.5mL
  – Aspirates: RTT, 1mL of saline + 0.1mL serum
  – Cavity fluid: Possibly EDTA and RTT one tube each, 400mL + few drops of serum (if TP <4mg/dl)

• Send all samples overnight with a cold pack

• Do not FREEZE samples!
Flow cytometry

• Cannot be run on serum or slides
• Samples need to be fresh
  – Test needs to be performed within 48 hours after obtaining the sample
• Is an interpretive test, so it is not just positive or negative
• Can often provide prognostic information
PARR

• PARR = PCR for antigen receptor rearrangement
• Every naïve B cell or T cell has its unique BCR or TCR
• PARR detects the monoclonal expansion of neoplastic lymphocytes
• Distinguishes neoplastic from inflammatory lymphoid cells
V(D)J recombination

Canine immunoglobulin gene loci have 80 V region genes and pseudogenes, 6 D genes and 6 J genes

BCR

TCR

V(D)J recombination

Nature Reviews | Immunology
Because of this V(D)J recombination, B cells and T cells have unique length of immunoglobulin and TCR gene
PARR assay

Because neoplastic lymphocyte expansion is monoclonal vs lymphoid hyperplasia is polyclonal...

Burnett RC et al, Vet Pathology 2003
PARR assay

Factors that can affect the results

• DNA quality (formalin)
• Presence of Taq inhibitors (formalin, mast cells, hemoglobin)
• PCR protocol
• Primer choice
• Electrophoresis (separation of DNA products)
PARR – sample submission

• **Blood/bone marrow**: EDTA tube, >200µL of sample
• **Aspirates**: slides, or EDTA tube with No added fluid
• **Cavity fluid**: EDTA tube, or on a slide
PARR – sample submission

• 50,000 cells are needed to be diagnostic
• If there are not enough cells, test result will come back non-diagnostic
  – Make sure your samples are cellular!
  – Stain the slides that you are submitting to make sure you have a good sample
• Samples do not need to be shipped overnight
• Samples submitted for cytology can be used for PARR
PARR

• CSU will not run PARR on formalin fixed/paraffin embedded samples
  – VDx will perform PARR on biopsy samples
• Sensitivity=75% for dogs, 65% for cats; Specificity >90% for dogs and cats
• Result will be positive or negative
Molecular Remission

• To evaluate whether the patient is in remission by performing PARR assay
• Sample: peripheral blood
• Can be performed:
  – Prior to consolidation therapy or stem cell transplantation
  – Before discontinuing chemotherapy
  – Predicting relapse?
PARR vs Flow cytometry

• Lymph node/organ aspirate
  – confirmed or strongly suspicious of lymphoma: *Flow cytometry*
  – heterogenous or reactive with rare suspicious cells: *PARR*

• Flow can give us more information than PARR
PARR vs Flow cytometry

• Peripheral lymphocytosis: Flow cytometry
• Pleural/peritoneal fluid
  – Large number of abnormal lymphoid cells – Flow cytometry
  – Rare abnormal lymphoid cells: PARR
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Case 1 - Maggie

• 11 yr FS Lab mix
• Presentation: Generalized lymphadenopathy
• Cytology of the peripheral nodes: Lymphoma
PARR or Flow?

• Lymph node/organ aspirate
  – confirmed or strongly suspicious of lymphoma: *Flow cytometry*
  – heterogenous or reactive with rare suspicious cells: *PARR*
Case 1 - Maggie

- Flow cytometry – B cell lymphoma
  - CD21/CD22 positive
  - Medium sized cells; high class II MHC expression
Case 1 - Maggie

• Flow provided phenotype and prognostic information
  – Medium sized cells and high MHC class II expression
    = Better prognosis 😊 (Rao S et al JVIM 2011)
  – Younger dogs (7 yr) with large sized cells, low class II expression mortality at 6 month more than 90%... 😞
Case 2 - Lola

• 9 yo FS Golden Retriever
• Presentation: Mandibular and parotid lymphadenopathy with peripheral lymphocytosis
• Cytology of the mandibular lymph node: possible early lymphoma
PARR or Flow?

• Lymph node/organ aspirate
  – confirmed or strongly suspicious of lymphoma: *Flow cytometry*
  – homogenous population of small lymphocytes: *Flow cytometry*
  – heterogenous or reactive with rare suspicious cells: *PARR*
Case 2 - Lola

• PARR
  Clonal T cell population

• Flow Cytometry
  CD4-CD8-CD5+CD45-: indolent T cell lymphoma
Case 2 - Lola

• GR with lymphadenopathy and lymphocytosis with CD45- T cell lymphoma
  = good prognosis, median survival of 637 days 😊
  (Seelig DM et al JVIM 2014)
Case 3 - Sadie

• 12 yr FS Golden Retriever
• Presentation: Generalized lymphadenopathy with peripheral lymphocytosis
• Cytology of mandibular lymph node: reactive lymph node vs early lymphoma
PARR or Flow?

• Lymph node/organ aspirate
  – confirmed or strongly suspicious of lymphoma: *Flow cytometry*
  – heterogenous or reactive with rare suspicious cells: *PARR*
Case 3 - Sadie

• Flow Cytometry
  – CD21 positive (B cell lymphoma)
  – Medium sized cells; class II MHC expression not noted
Case 4 - Douglas

- 7 yr MN Basset Hound
- Presentation: R popliteal lymphadenopathy, liver mass and sublumbar lymphadenopathy
- Cytology of the liver: lymphoma, high grade
PARR or Flow?

• Lymph node/organ aspirate
  – confirmed or strongly suspicious of lymphoma: *Flow cytometry*
  – heterogenous or reactive with rare suspicious cells: *PARR*
Case 4 - Douglas

PARR: negative
Case 4 - Douglas

• What does negative PARR mean?
  – Not lymphoma
  – PCR condition
  – NK cells (rare)
Case 5 – Sammy

• 11 yo MN Chihuahua mix
• Lymphocyte count: 72,300 cells/µL (small lymphocytes)
• Lymphocytic leukemia suspected
Case 5 – Sammy

• Flow Cytometry
  – CD21/CD22 positive (B cell)
  – Small lymphocytes

• Bone marrow not required for diagnosis of CLL!
Case 5 – Sammy

• B cell lymphocytosis, small cells
  – good long term prognosis of >1,000 days 😊
    Presenting lymphocyte count does not affect the survival time
  – CD21+ lymphocytosis composed of large cells have much shorter survival times of 129 days
    (Williams MJ et al, JVIM 2008)
Case 5 – Sammy

• T cell lymphocytosis, small cells
  – Survival times for Lymphocyte count of <30,000 cells/µL vs >30,000 cells/µL = 1,098 days vs 131 days (Williams MJ et al, JVIM 2008)
  – Survival times for CD3+CD8+ CLL vs atypical T cell CLL = 930 days vs 22 days (Comazzi S et al, JVIM 2011)
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Monoclonal antibody therapy

• Form of immunotherapy that uses antibodies to specifically bind to specific cells or proteins (wiki)

• More than 30 monoclonal antibodies are now FDA-approved

• Immunotherapy is currently the hottest topic in cancer research
How Rituximab works

- Antibody-dependent cell-mediated cytotoxicity
- Antibody-dependent cellular phagocytosis
- Complement-Dependent Cytotoxicity
Rituximab

• Brand name: Rituxan
• CD20 humanized (chimeric) antibody
• Breakthrough in treating numerous lymphoid proliferative diseases
• Has been used both in combination with chemotherapy or Rituximab alone
  – Induction, maintenance and rescue
Rituximab

Group d’Etude des Lymphomes de l’Adulte (GELA) – first study to show the benefit of Rituximab

2 year survival – 70 % vs 57 %
Rituximab – side effects

Side effects are usually mild but serious side effects can occur

• Infusion related reaction
  – Fatal infusion reactions may occur within 24 hours of Rituximab infusion
  – 80% occurred with first infusion

• Acute tumor lysis syndrome (rare)
Canine monoclonal antibody

• CD20 antibody
  – CD20 is a glycoprotein expressed exclusively on mature B cells
  – Granted full approval of licensure on Jan 1, 2015

• CD52 antibody
  – CD52 is a glycoprotein highly expressed on both B and T cells
  – Granted conditional licensure in Jan 2015
Canine monoclonal antibody

- CD20 and CD52 caninized (chimeric) antibody
- Available only in a limited number of sites nationwide
- Limited information available
- Release to other institutions unknown at this time
- Not available at ASG
Canine CD52 antibody

• Multicenter, randomized, placebo-controlled study of CD52 mAb in combination with CCNU chemotherapy currently underway
Canine CD20 antibody

Two pilot studies were presented at VCS

- Double-blind, randomized placebo-controlled study of CD20 mAb+L-CHOP
  - mAb + L-CHOP vs L-CHOP alone
  - Median PFS: 167 days vs 93.5 days
  - OST: 177 days vs 325 days
  - Adverse events were restricted to L-CHOP cycle
Canine CD20 antibody

- Open-label pilot study of Doxorubicin + CD20 mAb
  - mAb + DOX vs DOX alone
  - Median PFS: 98 days vs 57 days
  - Remission rate: 75% vs 40%
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Other new therapeutics under investigation

Tanovea (Rabacfosadine)

– Novel anticancer prodrug; has shown efficacy toward B and T cell lymphoma
– Has been shown to be effective with an overall response rate of 79%

Lawrence, et. al.: *Veterinary Radiology & Ultrasound* Vol. 50, No. 6, 2009, pp 660-668
Other new therapeutics under investigation

VDC-597 (PI3K and mTOR inhibitor)

— PI3K and mTOR signaling regulates cell growth, cell survival and cell movement
— Over-activation of PI3K and mTOR signaling has been reported in many types of cancer
— Clinical trial currently underway
Thank you!