Lymphoma & histiocytic sarcoma-
an update

Aleksandra Marcinowska
Lymphoma

- 30% of feline malignancies
- 10% of canine malignancies
LYMPHOMA

Many different disease entities!
Many different subtypes!
Classification based on morphology and immunophenotype (updated Kiel classification)
Grading determined by cell size and MI
No difference between age, sex.
Boxers predisposed
Nodal involvement: 82.4%, extra-nodal: 17.6%
388 cases-B cell, 143-T cell

Biopsy specimens from 992 dogs with clinical diagnosis of lymphoma. All immunophenotyped with CD79a and CD3, CD18. Describe 27 different entities!!
“Modified WHO criteria based on morphology and immunophenotype”
Lymphoma- to confuse matters more...

Indolent, low grade lymphomas traditionally expect long survival times with or without treatment. But can transform to higher grade malignancy resulting in a sudden acceleration of the disease. Management- monitoring of such cases. Place of marginal zone lymphomas.
Canine Lymphoma – anatomical classification

Multicentric [80 - 85%]

Mediastinal [5%]

Alimentary [5 - 7%]

Cutaneous [6%]

Extranodal [small %]
Feline Lymphoma – anatomical classification

Multicentric [15 - 20%]

Mediastinal [30%]

Alimentary [30 - 40%]

Cutaneous [rare]

Extranodal [25 - 30%]
Multicentric lymphoma

Most common form in dog:
• middle aged animals - 6 - 10 years
• no sex predilection
• boxers, bulldogs and bullmastiff at increased risk

Less common form in cat?
• middle aged animals
• ? % FeLV positive
Multicentric lymphoma

- Gross enlargement of 1+ lymph nodes
- May progress to involve other organs:
  - Spleen
  - Liver
  - Lungs
  - Bone marrow
Lymphoma – staging & diagnosis

- „gold standard“:
- Bloodwork (haematology, biochemistry)
- 2/3-view thoracic radiography
- Abdominal ultrasound
- Lymph node aspirates/biopsy
- Bone marrow aspirates
- Phenotyping- by means of flow cytometry or biopsy (also PARR)

? Basic amount of tests deemed sufficient in order to comfortably treat?
Multicentric lymphoma
Multicentric lymphoma
Multicentric lymphoma
Canine multicentric lymphoma

Clinical Staging subsets a (well) & b (poorly)

Stage 1 single node or lymphoid tissue in a single organ
Stage 2 regional nodal involvement +/- tonsils
Stage 3 generalized lymphadenopathy
Stage 4 + hepatic and/or splenic involvement
Stage 5 manifestation in blood, bone marrow & other organs
How do we stage canine lymphoma today: resolving or complicating the controversy?
Laura Marconato, Centro Oncologico Veterinario, Italy
Marconato 2010

• Comprehensive clinical history and physical examination

• Baseline laboratory analysis

• Bone marrow debatable. Bone marrow may not correlate with haematological abnormalities and vice versa

• Use of flow cytometry for diagnosis/staging, treatment monitoring and to detect residual disease
Flow cytometry

- Cytology vs histopathology
- Excisional biopsy in human classification
- Cytology—well established tool for diagnosis in humans
- Cytology—controversial in human NHL
- Cytology—useless for some, brilliant for others
Flow cytometry

Currently FNA followed by:

- Immunophenotyping
- Southern blotting
- PCR (PARR)
- FISH
- Laser scanning cytometry
- Microarrays
Flow cytometry

• Fundamental tool for diagnosis of haematological diseases

• Measures very large number of cells

• Records multiple parameters for individual cell

• Easy, quick results, cost comparable to HP

• Samples need to be shipped in a specific way in a short time space
Application of flow cytometry

• Lineage assessment

• Assessment of tumour maturation

• Antigen quantitation

• Tumour clonality

• Tumour staging

• Aberrant patterns

• Minimal residual disease
Management of multicentric lymphoma

- Establish diagnosis
- Establish extent (staging)
- Investigate complications
Is chemotherapy treatment justified in animals with lymphoma?

- CR / PR = essentially normal animal
- Side effects are minimal at dose rates used
- Requires sympathetic owner counselling & support and careful patient monitoring
- Can be very rewarding for all concerned!
Treatment of canine multicentric lymphoma - what works best?
Treatment of canine multicentric lymphoma

• No treatment:
  – mean survival 6 - 8 weeks

• Prednisolone only:
  – mean survival 2 - 3 months

• Chemotherapy:
  – mean survival 6 - 18 months, depending on protocol

• (Surgery / Radiotherapy)
Chemotherapy protocols for lymphoma

- **COP / LOP**
  - Cyclophosphamide / Lomustine
  - Vincristine
  - Prednisolone

- **COPA or CHOP**
  - Plus doxorubicin

- **VLCAP / UW-Madison**
  - Plus L-Asparaginase

- **Single agent**
  - Doxorubicin
Response - Canine Multicentric lymphoma

- 75 - 95% of dogs achieve remission
- Median remission: 3 - 12 months
- Median survival: 6 - 18 months

Protocols containing doxorubicin may be more effective
Treatment of feline lymphoma

- Fewer cats achieve CR

- But of those that do - longer survival

- Siamese cats may have more favourable prognosis

- FeLV +ve worse prognosis
Actions of cytotoxic drugs

NEOPLASTIC TISSUE

DIVIDING CELLS → SENSITIVE

RESTING CELLS → NO EFFECT
Actions of cytotoxic drugs

NORMAL TISSUE

DIVIDING CELLS

TOXICITY

RESTING CELLS

NO EFFECT
How are cytotoxic drugs administered?

- Use highest possible doses
- Single treatments not effective
- Combination of drugs from different groups
How are cytotoxic drugs administered?

- Cytotoxic cell kill is by first order kinetics

  *The number of cancer cells killed by a drug is proportional to the dose*

  \[ \text{Log no Tumour cells} \]

  \[ \text{time} \]

Therefore use the highest dose possible
How are cytotoxic drugs administered?

- Normal tissue toxicity constrains maximum dose
How are cytotoxic drugs administered?

• A single dose is unlikely to be effective

Therefore repeated treatments needed, timed to allow normal tissue recovery
How are cytotoxic drugs administered?

• Combination protocols
  – Additive tumour cell kill
  – Different normal tissue toxicity
• E.g.
Phases of Chemotherapy

- **INDUCTION**
- **MAINTENANCE**
- **RESCUE**

- **remission**
- **relapse**

**Limit of detection**
Toxicity - “side effects”

• Immediate

• Acute

• Late / cumulative
Immediate toxicity: hypersensitivity

- L-Asparaginase
- Doxorubicin

Route of administration:
- Pre-medicate with anti-histamine/dexamethasone
Acute toxicity: extravasation

- Doxorubicin
- Vincristine
Acute toxicity: extravasation

- Use I.V. catheter
- Flush with SALINE pre & post infusion
- Monitor vein during infusion
Toxicity

Remember that chemotherapeutics also affect ‘normal’ dividing cells esp:

– Bone marrow

– Gastrointestinal tract
Acute toxicity: myelosuppression

Most drugs

- Neutropenia → sepsis
- Thrombocytopenia → bleeding
- Anaemia → rarely a problem
Acute toxicity: gastrointestinal

- Anorexia, vomiting, diarrhoea
- Many drugs
- Symptomatic therapy
- Anti-emetics: metoclopramide, maropitant, ondansetron
- Appetite stimulants: cyproheptadine, (prednisolone)
Toxicity: Cyclophosphamide

Haemorrhagic cystitis

• Administer drug in morning
• Ensure good fluid intake
• Check urine regularly
Toxicity: Doxorubicin

• Cardiomyopathy
• Administer drug slowly
• Monitor ventricular fractional shortening
Late toxicity: hair loss

- Does not occur in all dogs

- Dogs with continuously growing hair coats (poodles, terriers, OES) loose hair

- Clipped hair regrows slowly

- Cats loose guard hairs and whiskers

- Hair regrows off chemo, but may vary in colour and texture
Cytotoxic drugs : Safety

• Risks from cytotoxic drugs
  – Inhalation
  – Ingestion
  – Skin contamination / absorption

• Tablets

• Injectables
Cytotoxic drugs : Safety

• Most cytotoxic drugs are mutagens and carcinogens, some may be teratogens

• Great care must be taken in the preparation and administration of these agents

• Local rules should be established for their safe handling
Cytotoxic drugs: Safety - **tablets**

- NEVER break or crush
- Clear labelling
- Child proof containers
- Clear instructions on administration
- Wear gloves to handle
- Not to be handled by pregnant women
Cytotoxic drugs: Safety - injectables

- Protective clothing
- Reconstitution
- Administration
- Waste disposal
- Spillage procedures
Cytotoxic drugs: protective clothing
Cytotoxic drugs: reconstitution
Cytotoxic drugs: administration
Cytotoxic drugs: administration
Administration

DOXORUBICIN

VINCristine
Cytotoxic drugs: waste disposal
Cytotoxic drugs: spillage
LOCAL RULES & PRACTICES
FOR
THE SAFE HANDLING OF CYTOTOXIC DRUGS IN THE
DEPARTMENT OF CLINICAL VETERINARY MEDICINE

(Revised May 2002)
Conclusions 1

• Canine malignant lymphoma should no longer be regarded as one disease entity

• There are many (>20) different subtypes with different clinical behaviour and therapeutic response

• Some subtypes are well defined, some are still to separate into more precise subtypes
Conclusion 2

• Whilst some sub-types may be diagnosed by cytology with flow cytometry

• Histopathology is necessary for those sub-types that require a structural evaluation of nodal or splenic architecture

• There is a strong case for lymphadenectomy to confirm and better characterise a primary cytological diagnosis
Conclusion 3
There is no consensus on classification between Europe & US, however a prognostically relevant theme seems to be developing with three main groups:

• Diffuse large cell B cell lymphoma (DLBCL) is the most common form of lymphoma in the dog

• High grade, lymphoblastic, peripheral T cell lymphomas form another group with generally poor prognosis
Histiocytic sarcoma (HS)

The dendritic cell lineage and immunophenotype of canine histiocytic diseases

Key:
- CD = Cluster of differentiation
- MHC II = Major histocompatibility complex II
- Thy1 = Thymocyte differentiation antigen 1
HS overview

• Most canine and feline histiocytic tumours involve proliferation of Langerhans cells (LC) or interstitial dendritic cell (DC) lineage

• Hemophagocytic histiocytic sarcoma is the only histiocytic disease of dogs and cats that originates from macrophages
<table>
<thead>
<tr>
<th>Disease</th>
<th>Species</th>
<th>Cell of origin</th>
<th>Key morphological feature</th>
<th>Immunophenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histiocytoma</td>
<td>Dog</td>
<td>LC</td>
<td>Lesions-epidermal focus, intraepidermal foci-common. Histiocytes- diverse nuclear morphology. Multinucleated cells and cytologic atypia-rare.</td>
<td>CD1a, CD11c/CD18, E-cadherin</td>
</tr>
<tr>
<td>Cutaneous Langerhans cell histiocytosis</td>
<td>Dog</td>
<td>LC</td>
<td>Multiple cutaneous lesions observed. Metastasis to lymph nodes and internal sites-possible. Lesions otherwise identical to histocytoma, may have higher frequency of multinucleated cells and cytologic atypia.</td>
<td>CD1a, CD11c/CD18, E-cadherin</td>
</tr>
<tr>
<td>Pulmonary Langerhans cell histiocytosis</td>
<td>Cat</td>
<td>LC</td>
<td>Multinodular to diffuse involvement of all lung lobes. Lesions-cohesive histiocytic infiltrates, which obliterate terminal airways and extend to pleural surfaces.</td>
<td>CD1a, CD18, E-cadherin</td>
</tr>
<tr>
<td>Cutaneous histiocytosis</td>
<td>Dog</td>
<td>iDC activated</td>
<td>Vasocentric lesions focused on mid-dermis to subcutis. Lesions are pleocellular but are dominated by histiocytes and lymphocytes. Lymphohistiocytic vasculitis is commonly observed. Histiocytes lack cytologic atypia and multinucleated giant cells are rare. Skin draining lymph node may be infiltrated.</td>
<td>CD1a, CD4, CD11c/CD18, CD90</td>
</tr>
<tr>
<td>Systemic histiocytosis</td>
<td>Dog</td>
<td>iDC</td>
<td>Lesions are identical to cutaneous histiocytosis in skin. Lesions extend to lymph nodes, ocular and nasal mucousa, and internal organs.</td>
<td>CD1a, CD4, CD11c/CD18, CD90</td>
</tr>
<tr>
<td>Histiocytic sarcoma (localized&amp;disseminated)</td>
<td>Dog, cat</td>
<td>iDC</td>
<td>Mass lesions are observed in spleen, lung, lymph node, and other primary tissues sites. Histiocytes are pleomorphic, mononuclear, and multinucleated giant cells with marked cytological atypia.</td>
<td>CD1a, CD11c/CD18</td>
</tr>
<tr>
<td>Histiocytic sarcoma-hemophagocytic</td>
<td>Dog, cat</td>
<td>Macrophage</td>
<td>Mass lesions are lacking. Diffuse splenomegaly and insidious infiltration of liver, lung and bone marrow are consistently observed. Splenic red pulp is expanded by erythrophagocytic histiocytes. Mononuclear and multinucleated giant cells with cytologic atypia are common.</td>
<td>CD1a (low), CD11d/CD18 (dog)</td>
</tr>
<tr>
<td>Feline progressive histiocytosis</td>
<td>Cat</td>
<td>iDC</td>
<td>Skin nodules and plaques are observed. Lesions occupy the dermis with an epidermal focus. Intraepidermal foci (40%) occur. In early lesions, histiocytes have minimal cytologic atypia. In later lesions, histiocytes manifest cytological atypia as in histiocytic sarcoma.</td>
<td>CD1a, CD11/CD18, CD5 (50%)</td>
</tr>
<tr>
<td>Dendritic cell leukemia</td>
<td>Dog</td>
<td>iDC</td>
<td>Predominant blood and bone marrow involvement observed. There is diffuse infiltration of spleen, lung, liver. Histiocytes manifest moderate cytologic atypia in blood and tissues.</td>
<td>CD1a, CD11c/CD18</td>
</tr>
</tbody>
</table>
HS complex

• Most often derived from cells with phenotypic profile of interstitial DCs

• HS may be localized or disseminated (the latter previously known as malignant histiocytosis)

• The HS complex first recognized in Bernese Mountain Dogs
HS complex- breed predisposition

Bernese Mountain Dog
Rottweiler

Flat-coated retriever
Golden retriever
HS – breed predisposition

Journal of Small Animal Practice

Histiocytic sarcoma in 14 miniature schnauzers- a new breed predisposition?
JA Lenz, E Furrow, LE Craig, and CM Cannon 2017

14 dogs, 10 localized, 4 disseminated
pedigree analysis supported an inherited risk for HS in breed
HS – breed predisposition

Pembrooke Welsh Corgie at risk for primary HS of the CNS

Also primary pulmonary involvement reported in this breed
HS complex- localized HS

• Clinical signs – often vague and nonspecific

• Primary lesions: spleen, lymph node, lung, bone marrow, CNS, skin, subcutis, **periarticular and articular tissues of the limbs**.

• Secondary sites-widespread: liver, lungs (with splenic primary) and hilar lymph nodes (with lungs primary)
HS complex- localized HS

• Lesions: intra-articular or peri-articular of the appendicular skeleton

• The most common tumour of joints in dogs; in cats occurs at much lower incidence

• Stifle and elbow joints most commonly affected

• History of anterior cruciate rupture/ traumatic injury to joint- associated with development of articular HS
HS complex- localized HS
HS complex- localized HS

- Complete staging- essential
- CBC & biochemistry: anemia, leukocytosis, thrombocytopenia, increased liver enzymes, hypoalbuminemia, hypocholesterolemia-frequent findings
- Hyperferritinemia reported- most likely as a result of ferritin production by tumour cells
- Thoracic radiographs and abdominal ultrasound: pulmonary involvement- diffuse, patchy, or focal. Hepatosplenomegaly, hepatic/splenic mottling and nodules-common
- Bone marrow?
HS complex- localized HS

• Diagnosis: cytology, histopathology, confirmed with IHC/immunocytochemistry on formalin-fixed tissue (CD18, MHC II)

• Fresh or frozen tissue can be used to further confirm and subclassify the cell of origin (CD1, CD11a)

• Differentiation between macrophage/ granulocyte, lymphoma
Figure 14. Tibiotarsal joint; dog; canine articular HS. Intra-articular and periarticular nodules, which have coalesced to form a mass.

Figure 15. Tibiotarsal joint; dog; canine articular HS. The tumor growth is discretely nodular with regional coalescence to form intra-articular nodular masses. The synovium is intact over these nodules.

(*Pictures taken from “A review of histiocytic diseases of dogs and cats”- P.F. Moore, Veterinary Pathology 2014*)
HS complex- localized HS

Figure 16. Tibiotarsal joint; canine articular HS. The synovium is intact above the tumor (arrow). There is a lymphoplasmacytic infiltrate immediately below the synovial membrane. A thin band of collagenous connective tissue (*) separates the synovial membrane from the tumor infiltrate that consists of histiocytes, which manifest cytological atypia, and interspersed, moderately intense lymphoplasmacytic inflammation.

Figure 17. Tibiotarsal joint; canine articular HS. The synovial membrane consists of CD18þ type A cells (arrow) and CD18– type B cells. Beneath the thin collagenous connective tissue band (*), the neoplastic histiocytes express CD18 in a contiguous pattern (pictures taken from “A review of histiocytic diseases of dogs and cats” - P.F. Moore, BVSc, Phd, DipACVP, Veterinary Pathology 2014)
HS complex- localized HS

- Periarticular location may be associated with better prognosis than other locations

- But...

- Metastatic rate high: 60% to over 90%
HS- treatment

- CCNU alone or in combination with surgery/radiotherapy

- Other chemotherapeutics: liposomal DOX, paclitaxel, cyclophosphamide, vincristine, prednisolone, mitoxantrone, dacarbazine, etoposide, toceranib phosphate?
HS – treatment

- RR to CCNU=26-46% (localized or systemic)

- Localized HS treated with a multimodal approach (radiotherapy and CCNU) - longer survival times (208 days) than dogs treated with surgery or radiotherapy alone (68 days). Long term survivals possible with radiation alone (5x6Gy) in localized form

- Surgery and CCNU (localized and visceral) 18.9 months in another report
HS- treatment

Australian Veterinary Journal
Chemotherapy for dogs with lymph node metastasis from histiocytic sarcomas
AS Moore, DP Taylor, G Reppas, and AE Frimberger 2017

12 dogs with lymph node metastases but no visceral metastases
• 8 treated with chemotherapy-MST 219d (77-1638); 1- and 2-year estimated survival rates were 37.7%
• 4 dogs not treated with chemotherapy – MST 57d (39-136), none alive 1 yr after surgery
• Various lomustine-based chemotherapy protocols
Thanks for listening!