An Update on Breed specific Cancer Research

Dr Jane Dobson

University of Cambridge

25 years of working with Flat-coats

- Tumour survey 1990 - 2015
- Defining sarcomas in the breed
- Mortality study
- Cause of Death Register

Histological Survey : 1990 - 2015

- Tumours / tumour like lesions from Flat-coated Retrievers
- Pedigree Information
- Free histopathology*

** Funded at cost by Flat-coated Retriever Owners & Breeders*

Results 1990 - 1998

(*Morris et al, Veterinary Record 2000; **147**: 291 - 295*)

PAPERS & ARTICLES

Histopathological survey of neoplasms in flat-coated retrievers, 1990 to 1998

J. S. MORRIS, D. E. BOSTOCK, E. F. MCINNES, T. M. HOATHER, J. M. DOBSON

Over the period from March 1990 to December 1998, veterinary surgeons in general practice were invited to submit tissues suspected of being neoplastic which had been removed from flat-coated retrievers. When possible, pedigree details were obtained from the owners. In addition, data were collected from flat-coated retrievers known to have suffered from a neoplastic condition and for which a histopathological report was available. A total of 1023 submissions was obtained from 782 dogs. These included 165 non-neoplastic lesions (16 per cent), 447 benign samples (44 per cent) and 411 malignant samples (40 per cent). Soft tissue sarcomas accounted for 55 per cent of the malignant samples (26 per cent of all tumour samples and 22 per cent of all submissions) with 63 per cent of them being diagnosed as undifferentiated. Carcinomas accounted for 20 per cent of malignant samples (8 per cent of all submissions). Of the benign tumours, cutaneous histiocytoma was the most common diagnosis (48 per cent of benign tumours, 25 per cent of all tumours and 21 per cent of all submissions).

Benign tumours

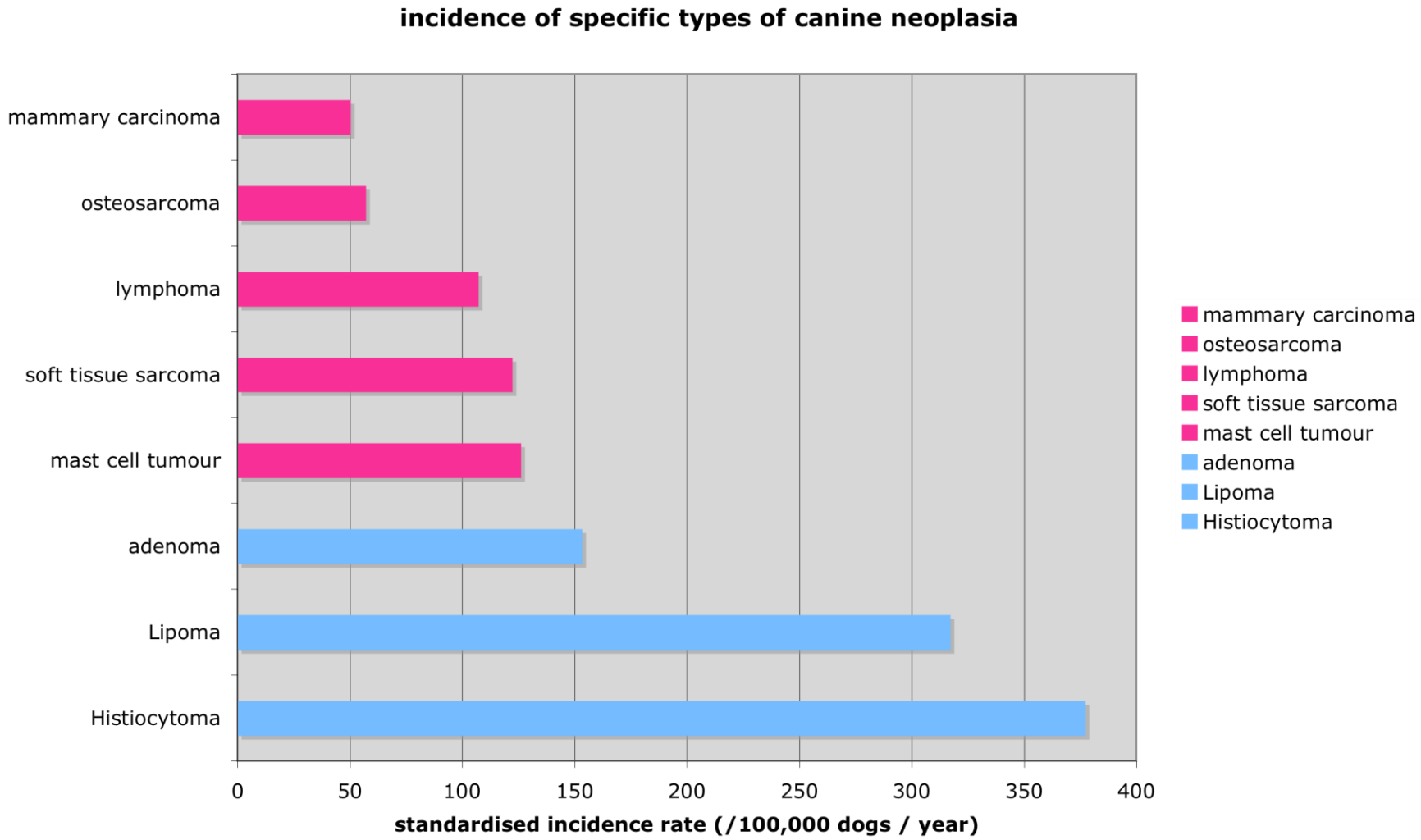
n = 446

Histiocytoma	216
Lipoma / fibrolipoma	84
Benign oral lesions	48
Haemangioma	17
Trichepithelioma	14
Papilloma	12
Mammary	11

Malignant tumours

n = 411

Sarcoma	225
Carcinoma	82
Lymphoma	30
Mast cell tumour	23



Dobson et al, JSAP 2002;43: 240 - 246

“Soft Tissue Sarcoma”

Term used to describe all malignant tumours of soft tissues

Common features:

- Histological features
- Clinical presentation
- Biological behaviour
- Therapeutic response

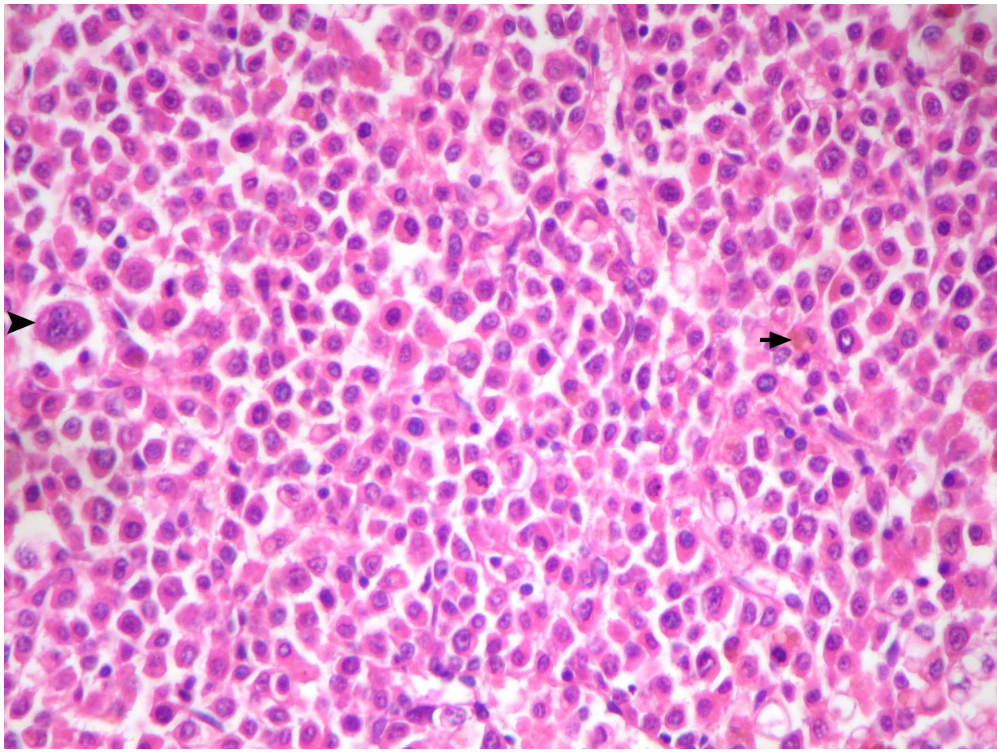
Breed Associations - Genetics

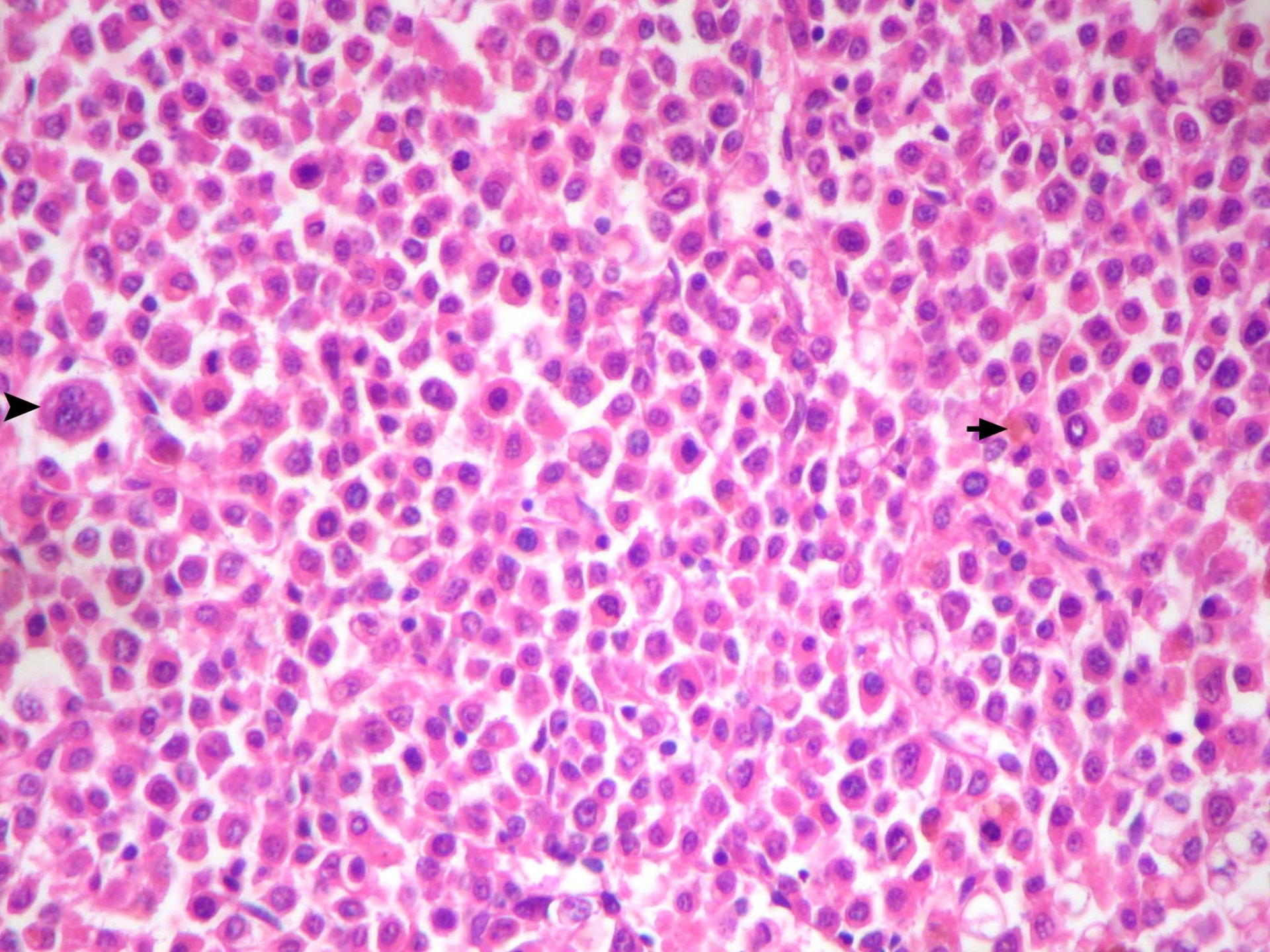
- Bernese Mountain Dogs - systemic & malignant histiocytosis
- German shepherd dogs - haemangiosarcoma
- Flat-coated retrievers - soft tissue sarcoma
- Labradors & boxers - mast cell tumours
- Giant breeds - osteosarcoma

Malignant Tumours of Soft Tissue

Poorly differentiated soft tissue tumours may lack features allowing them to be classified as specific tissue type hence:

- "Spindle cell" sarcoma
- "Round cell" sarcoma
- "Anaplastic" sarcoma





Soft tissue sarcomas in Flat-coats

Soft tissue sarcomas

= 55% of malignant tumours & 26% of all tumours

63% of soft tissue sarcomas in this series were "undifferentiated"

Commonly arise in the deep musculature of the limbs (49 fore, 34 hind)

Mean age 8 yrs (1 - 13 yrs)

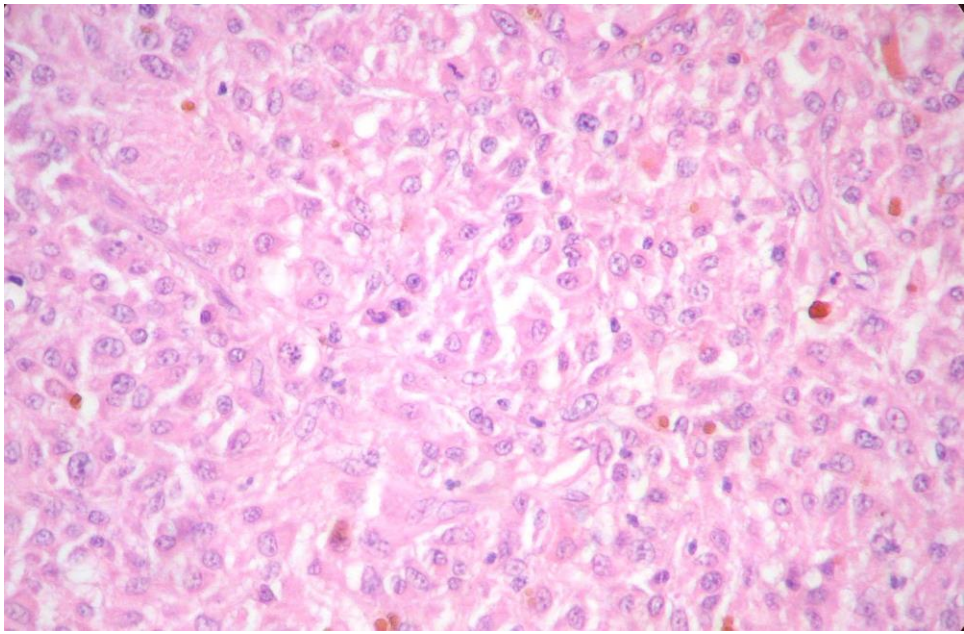
Immunohistochemical evaluation of 14 FC sarcoma

(Morris and others, Vet Pathol. 39: 473 - 479, 2002)

- Vimentin: all 100%
- Actin: 10 - 60%
- Desmin: 2 - 50%
- MHC Class II 70 - 90%

Significant myofibroblast component

Mild to moderate T cell infiltrate



These sarcomas belong to a spectrum of tumours which have features consistent with the diagnostic criteria for Malignant Fibrous Histiocytoma (MFH)

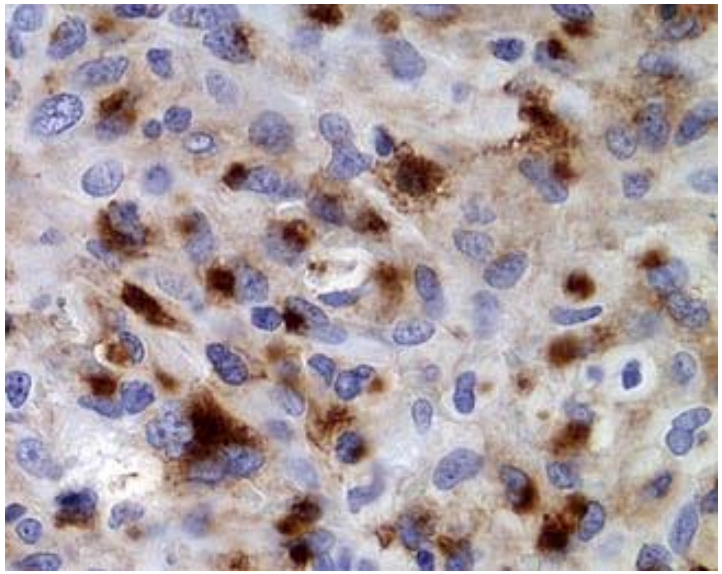
Vet Pathol **39**:473–479 (2002)

Immunohistochemical and Histopathologic Features of 14 Malignant Fibrous Histiocytomas from Flat-Coated Retrievers

J. S. MORRIS, E. F. MCINNES, D. E. BOSTOCK, T. M. HOATHER, AND J. M. DOBSON

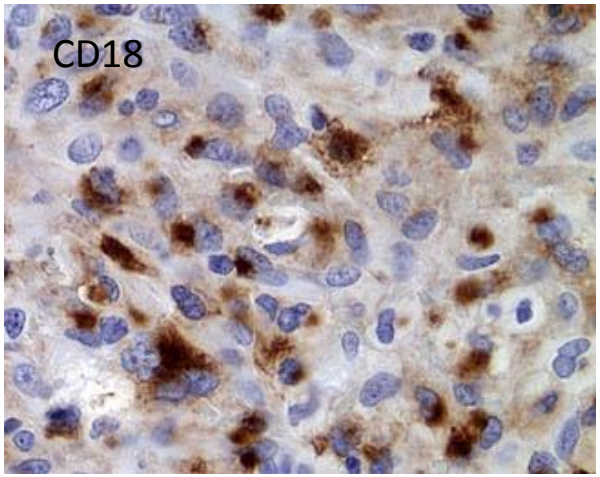
Department of Clinical Veterinary Medicine, University of Cambridge, Cambridge, UK (JSM, DEB, TMH, JMD); and Department of Pathology, Papworth Hospital, Papworth Everard, Cambridge, UK (EFM)

Abstract: Flat-Coated Retrievers seem to be at increased risk of developing soft-tissue sarcomas, and undifferentiated round cell or spindle cell sarcomas account for approximately 59% of sarcomas in the breed.



Changing terminology

Malignant Fibrous Histiocytoma (MFH) ⟷ Malignant Histiocytosis



Solitary lesions

Multifocal lesions

Localised Histiocytic sarcoma

Disseminated Histiocytic sarcoma

Affolter & Moore, Vet Pathol. 2002; 39(1): 74 - 83

Flatcoated Retriever Health Study 1996 - 2006

Recruited 174 healthy dogs

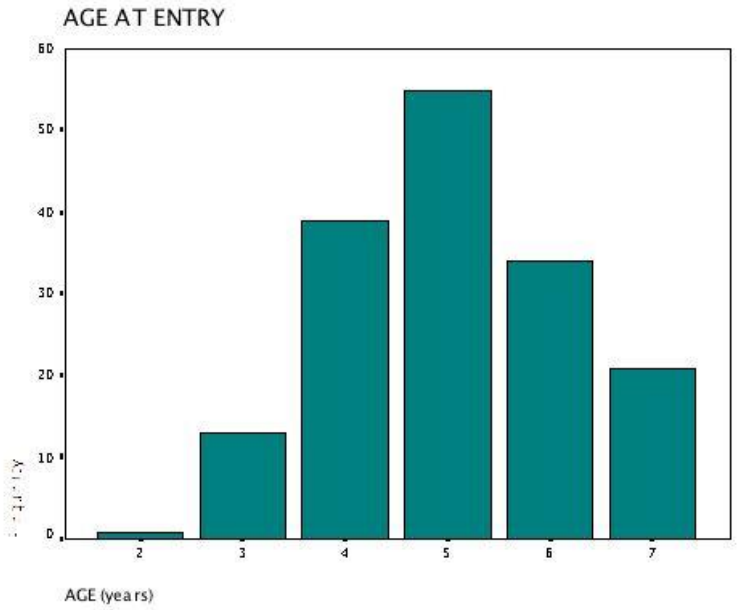
Age 2 - 7 yrs

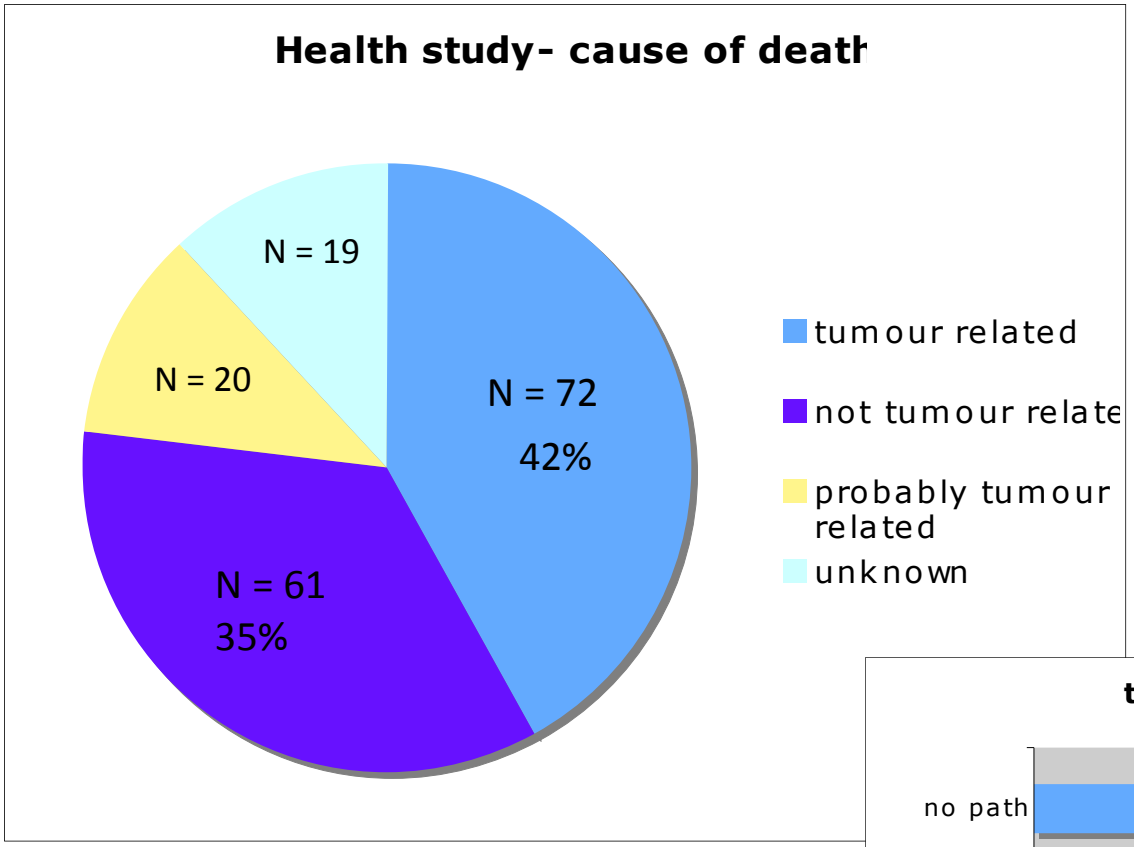
Pedigree details

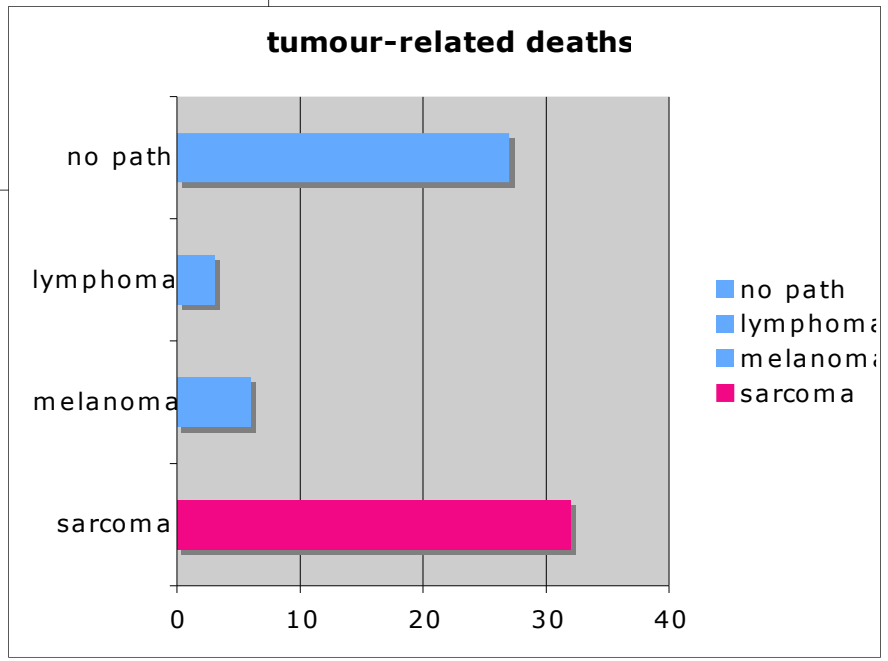
Annual health census

Results of Health study

- 174 dogs recruited
  - 2 totally lost to follow up
  - All others now dead
- Results based on 172 dogs

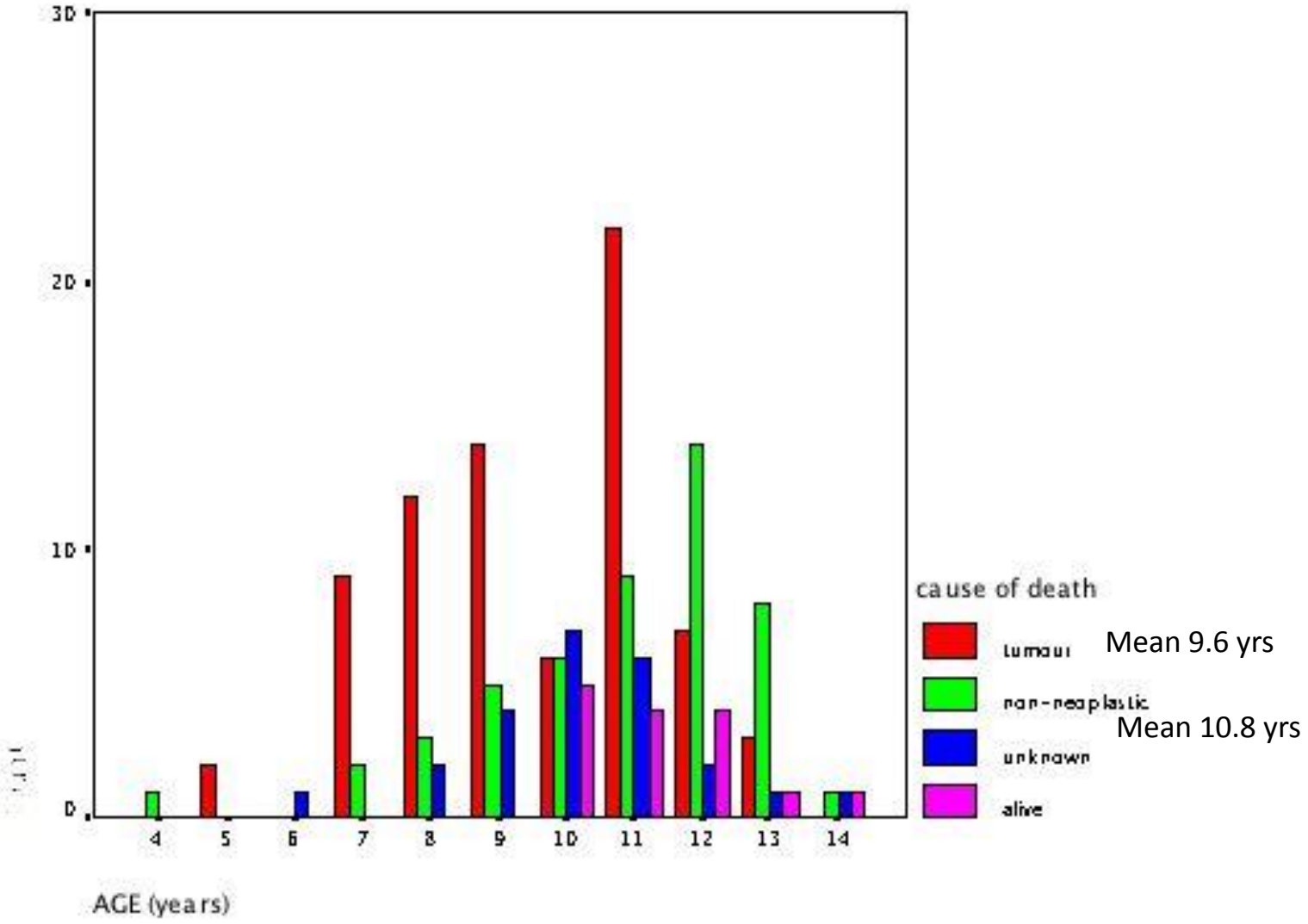






32 of 172 dogs [18%]
had histologically confirmed sarcoma

Age at Death



Mean 9.6 yrs

Mean 10.8 yrs

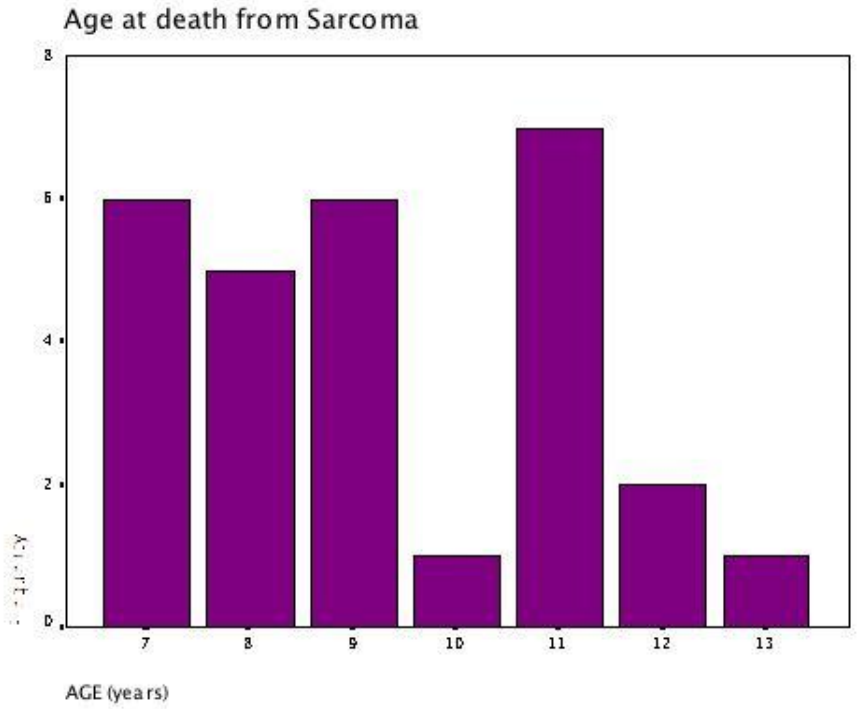
Tumours
n = 73 (42% of dogs)

- Sarcoma (ST) = 32
  - Anaplastic / undifferentiated = 22
  - Haemangiosarcoma = 4
  - Osteosarcoma = 5
- Malignant melanoma = 6
- Lymphoma = 3
- Others
  - MCT, mesothelioma,meningioma,mammary Ca, bile duct Ca
  - Liver, gastric, lung, spleen, brain, abdominal mass

- ? Tumour no pathology = 20

Soft tissue sarcoma

- 32 of 172 dogs
- 18.6 % of study dogs affected = 1.8 dogs in 10
- Age 7 - 13, mean 9.3 yrs

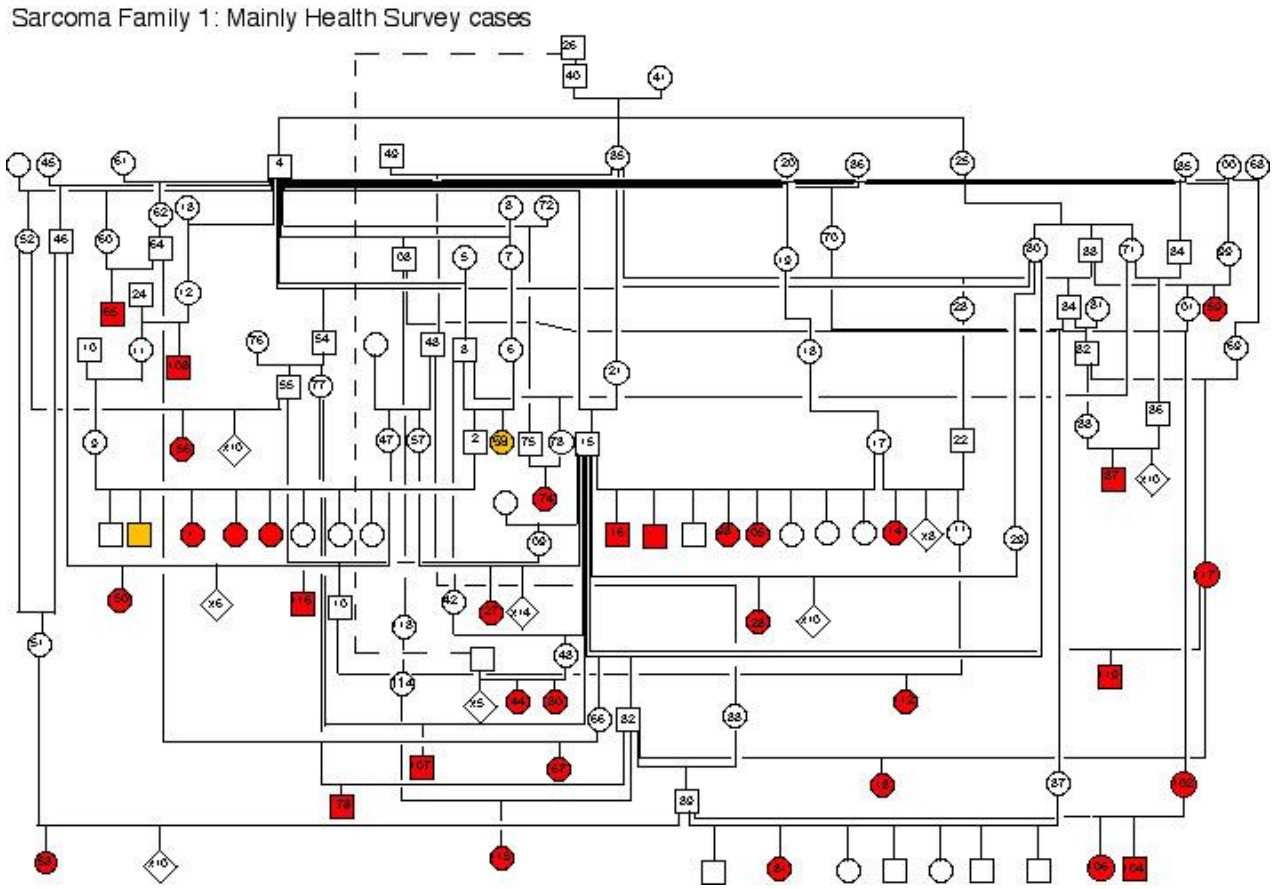


Non-neoplastic causes of death
n = 61 (35%)

- Heart disease = 14
- “Old age” = 12
- GDV = 3
- Arthritis/ hind limb weakness =11
- Laryngeal paralysis = 4
- Renal / liver failure = 8
- Intestinal obstruction, gastroenteritis, neurological, pyometra, disease, RTA, viral infection, Cushing’s disease, diabetes insipidus

Pedigree analysis

Jesus Aguirre-Hernandez



? An autosomal recessive inheritance with between 50 – 100% penetrance,
but there are other explanations for the patterns seen.
All affected dogs share 6 common ancestors (3M & 3F) 4 – 9 generations in the past

Case presentation

7 y.o. M Flatcoated Retriever

- 2 month history, progressive:
- Lethargy
- Reduced appetite
- Weight loss

Clinical findings

- Weight loss
- Pale mucous membranes
- Enlarged spleen on abdominal palpation

- Plan:
  - Blood for haematology & biochemistry
  - Ultrasound abdomen / spleen

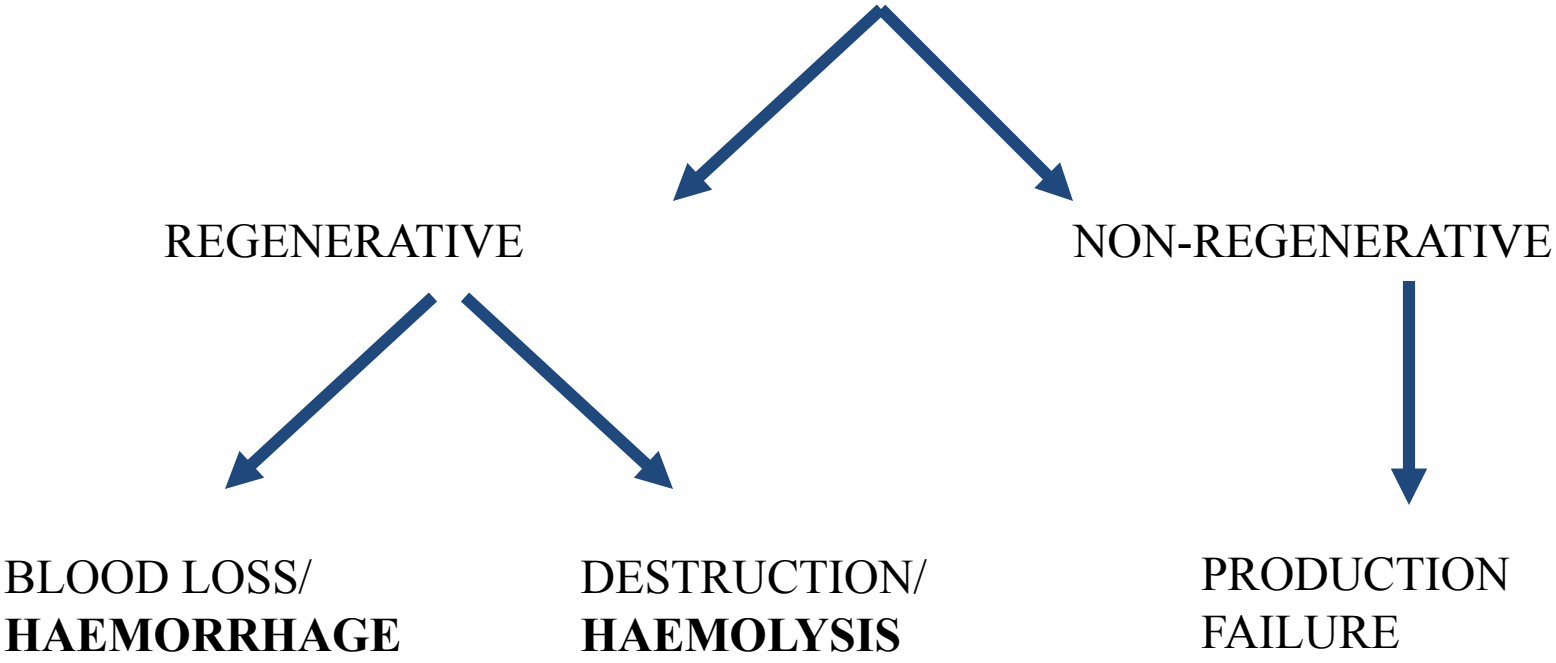
Haematology

	Patient		Normal range
Red Blood Cells	1.2	↓	5.5 – 8.5 x 10^{12}/l
Packed Cell volume	0.10	↓	0.37 – 0.55 l/l
Reticulocyte count	14 %	↑	< 50 x 10^{9}/l
Total WBC	51.3	↑	6 – 17 x 10^{9}/l
Monocytes	1		3 – 9 %
Platelets	4	↓	175 – 500 x 10^{9}/l
Total serum protein	28.6	↓	60 – 80 g/l
Albumin	9.4	↓	25 – 40 g/l
Globulin	19.2	↓	25 – 45 g/l

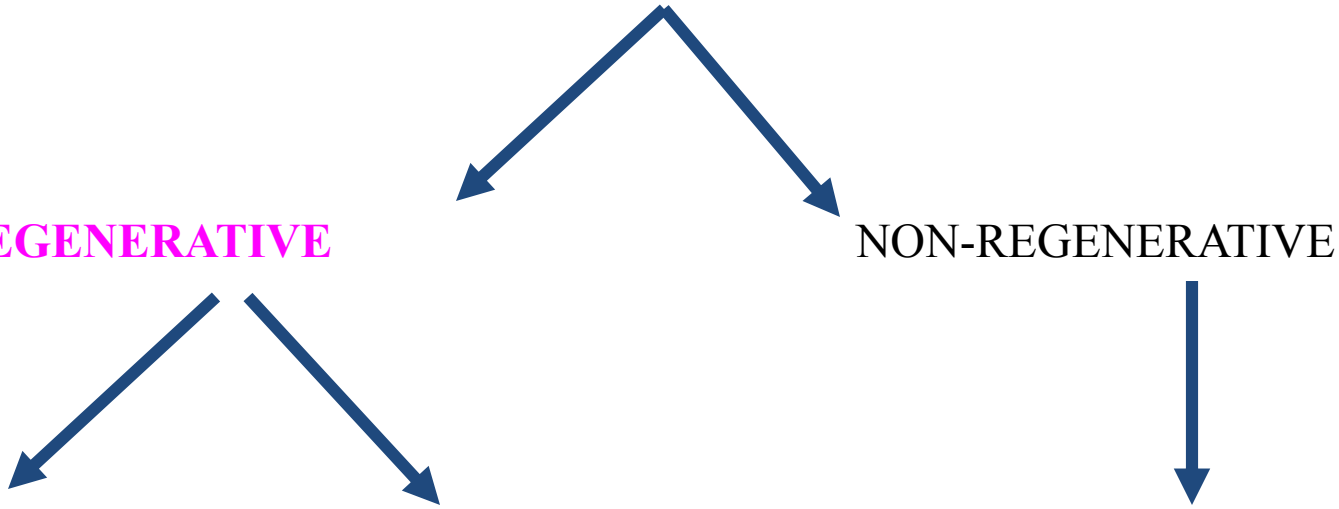
Major abnormalities

- Anaemia : regenerative, Coombs' negative
- Thrombocytopenia
- Hypoproteinaemia especially albumin
- Leucocytosis with extreme neutrophillia

Anaemia


REGENERATIVE
NON-REGENERATIVE
BLOOD LOSS/
HAEMORRHAGE
DESTRUCTION/
HAEMOLYSIS
PRODUCTION
FAILURE

Anaemia



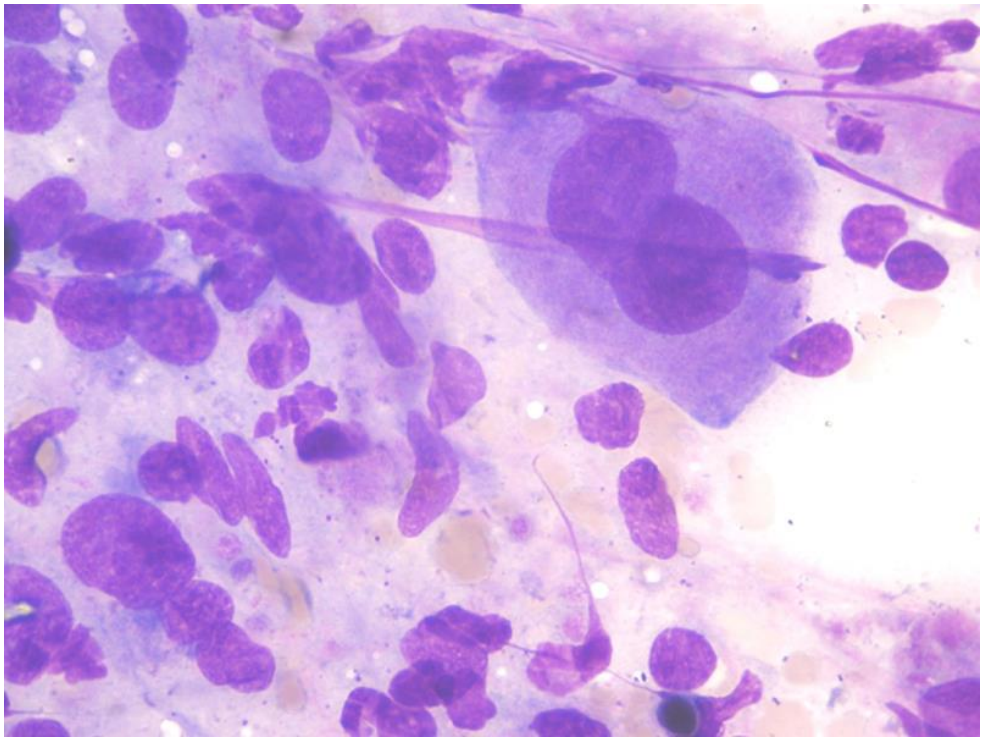
Low protein

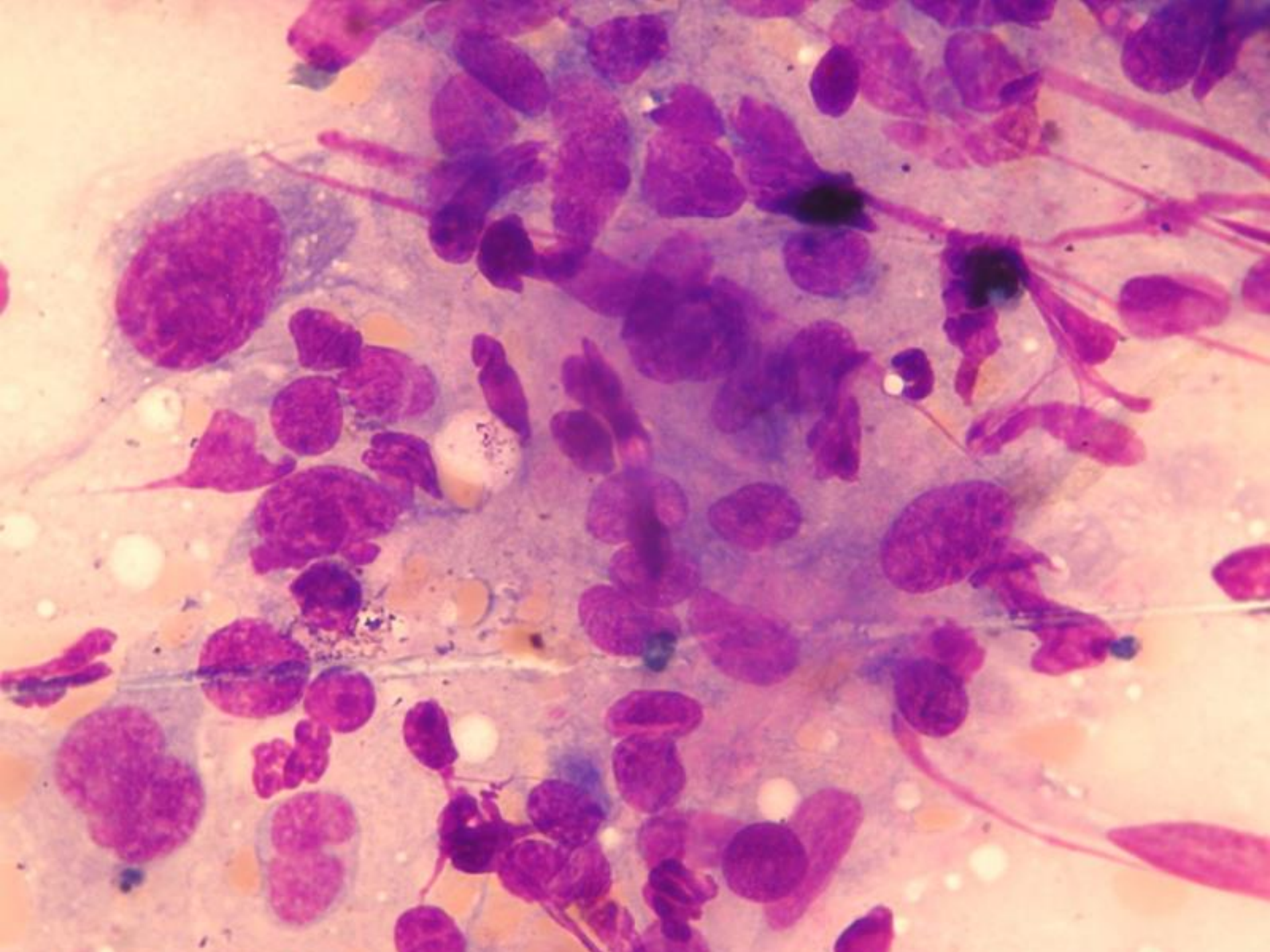
IMHA - Coombs' +

Ultrasound examination

FNA Cytology

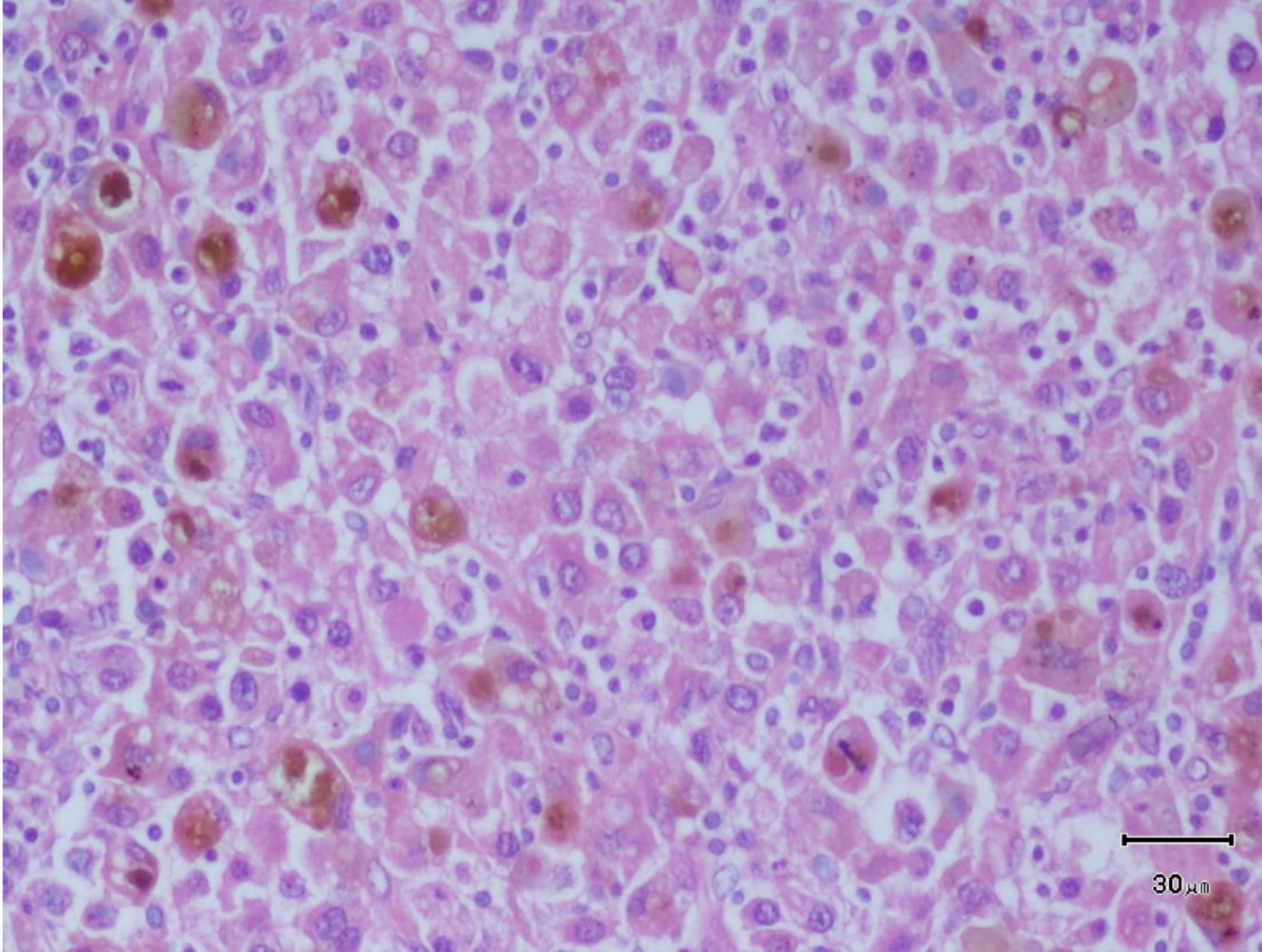
- Numerous large round cells in rafts
- Abundant basophilic cytoplasm with dense chromatin nucleus, large irregular prominent nucleoli
- Erythrophagia





Outcome

- Dog euthanased
- Post mortem examination:
  - **Gross** :enlarged spleen with irregular granular surface, diffusely infiltrated by disseminated cream to yellow nodules.
  - **Microscopic**: hypercellular coalescing infiltrates of polygonal cells with variable amounts of intracytoplasmic haemosiderin and evidence of erythrophagocytosis
  - **Diagnosis** : Histiocytic sarcoma of spleen


30 μm

4 very similar cases

(Dobson & others, Vet Rec 2006)

- 6.5 y.o. F(n) Flat-coated Retriever - PJW
  - Lethargy & anaemia - 6 weeks
- 8 y.o. M (n) Flat-coated Retriever - PM
  - Decreased appetite, lethargy & anaemia - 4 weeks
- 5y 9m F(n) Flat-coated Retriever - Survey
  - Lethargy, anaemia, inappetance
- 9 y 11m M Flat-coated Retriever - Survey
  - Anaemia

Vet Pathol **43**:632-645 (2006)

Canine Hemophagocytic Histiocytic Sarcoma: A Proliferative Disorder of CD11d+ Macrophages

P. F. Moore, V. K. Affolter and W. Vernau

Department of Veterinary Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, CA

17 dogs: BMD (6), golden retriever (4) rottweiler (3), Labrador retriever (2)
Coombs negative regenerative anaemia (16/17), thrombocytopenia (15/17)
Hypoalbuminaemia (16/17), hypocholersterolaemia (11/16).
Diffuse splenomegaly, ill-defined masses.
Microscopic lesions prevalent in spleen, liver, lung and bone marrow.
Origin, splenic red pulp and bone marrow macrophages, expressing MHC class II and CD11d.

Disseminated Histiocytic Sarcoma
(Malignant Histiocytosis)
Bernese Mountain Dog - frequency of approx 25%

Multifocal: lungs, spleen, liver and lymph nodes

Ramsey IK, McKay JS, Rudort H., Dobson JM, Malignant histiocytosis in three Bernese mountain dogs. Vet Rec (1996) 138: 440 - 444

Visceral HS in FCRs

Histiocytic sarcoma of the
Spleen in flat-coated retrievers
With regenerative anaemia
And hypoproteinaemia

Vet Rec (2006) 158: 825 - 829

Review site / presentation of “HS” in FCR-Cancer Survey 1999 - 2009

	male	female	total
Limb sarcoma	50	51	101
Visceral	26	21	47
Other Location	12	18	30
Histiocytic sarcoma	25	29	54
Other sarcoma	64	62	126
Total	89	91	180

Total submissions 1999 - 2009 = 1,600

Limb sarcomas

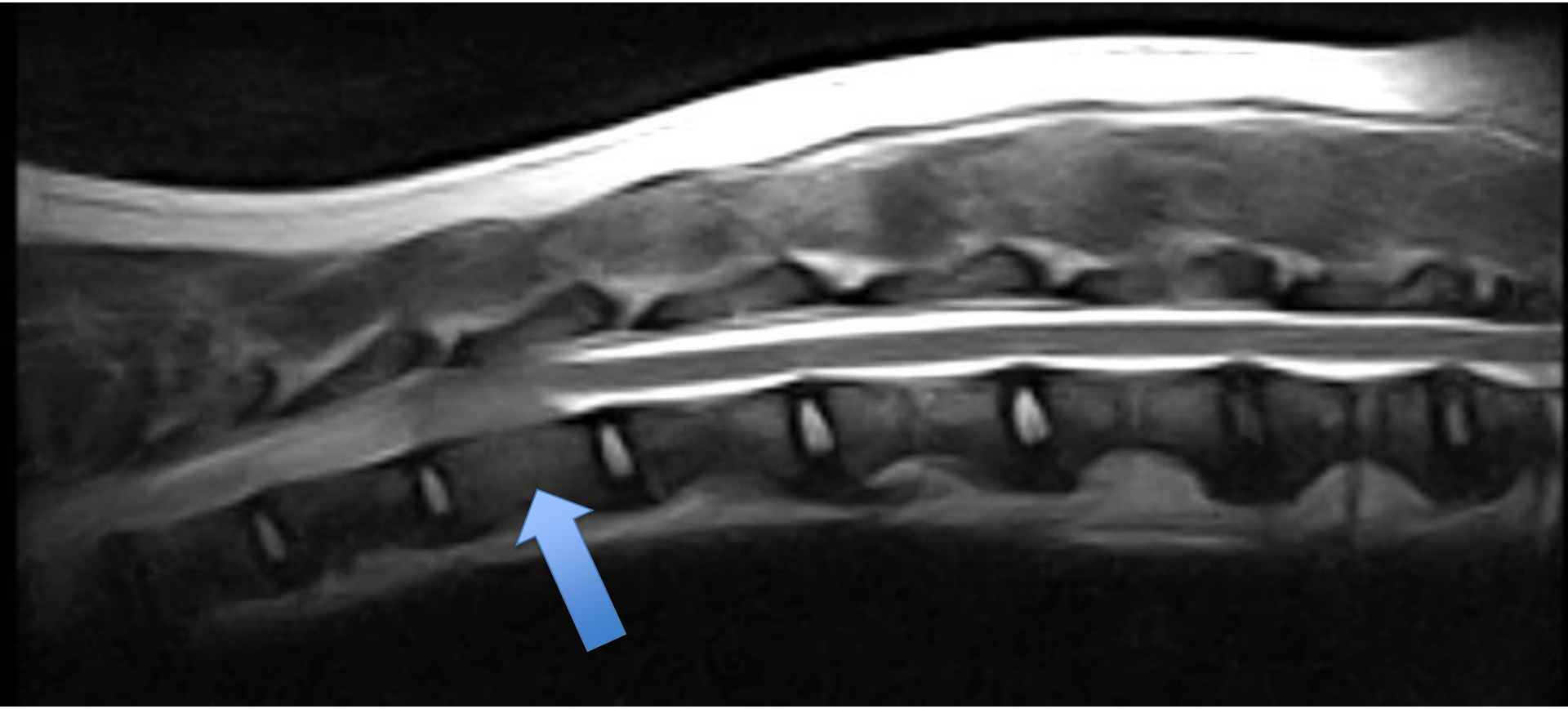
Site		number
Forelimb 80 lesions, 73 dogs	elbow	26
	shoulder	13
	brachium	9
	other	32
Hindlimb 28 lesions	stifle	10
	hip/pelvis	5
	other	13

Visceral Sarcomas

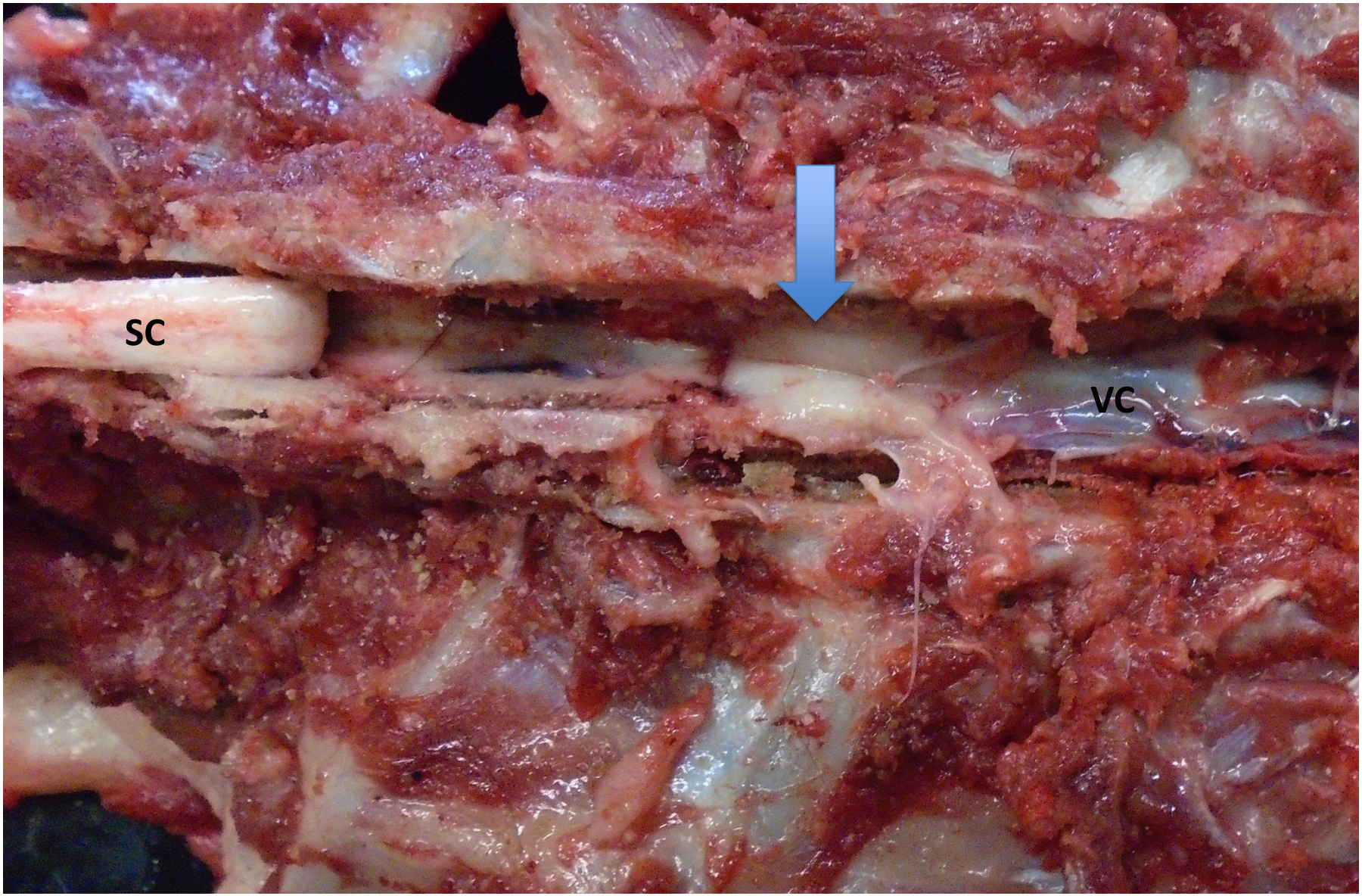
Site	Number of dogs	% visceral lesions (n = 47)
Spleen	31	66
Lung	10	21
Kidney	4	8.5
Liver	7	14.9
Lymph node	5	10.6
Bone marrow	1	-
GIT	6	12.8
Mediastinum / pleura	6	12.8

Other presentations: CNS

- 9 y.o. F. Flat-coated Retriever
- Sudden onset & progressive hind limb ataxia
- Radiographs NAD
- Neurological localisation to thoraco-lumbar spine, T 11 – L2
- MRI

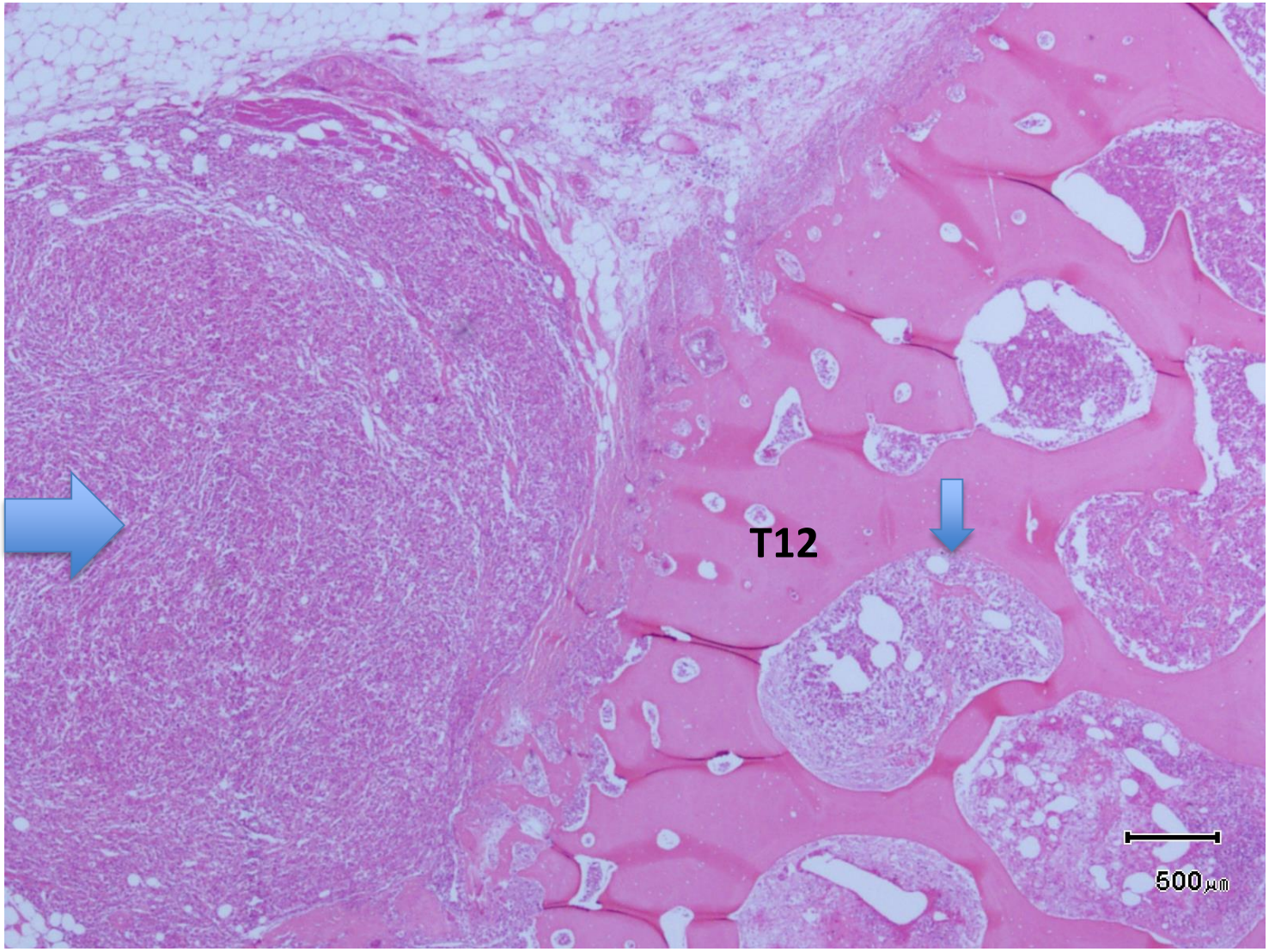


Space occupying lesion causing compression of the spinal cord

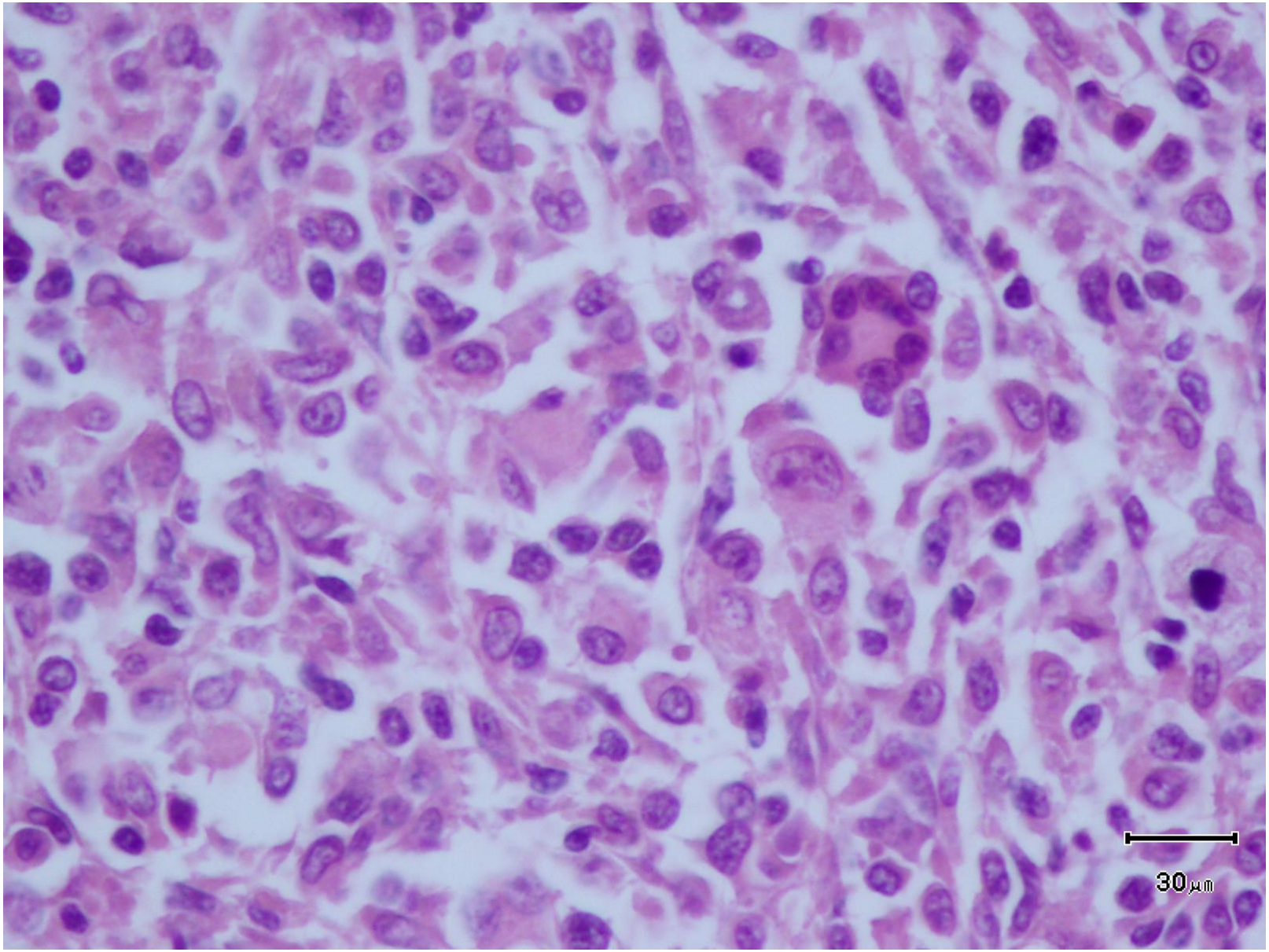


Vertebral (spinal) canal (VC) showing a mass (arrow) protruding from T12. The spinal cord (SC) has been retracted backwards.

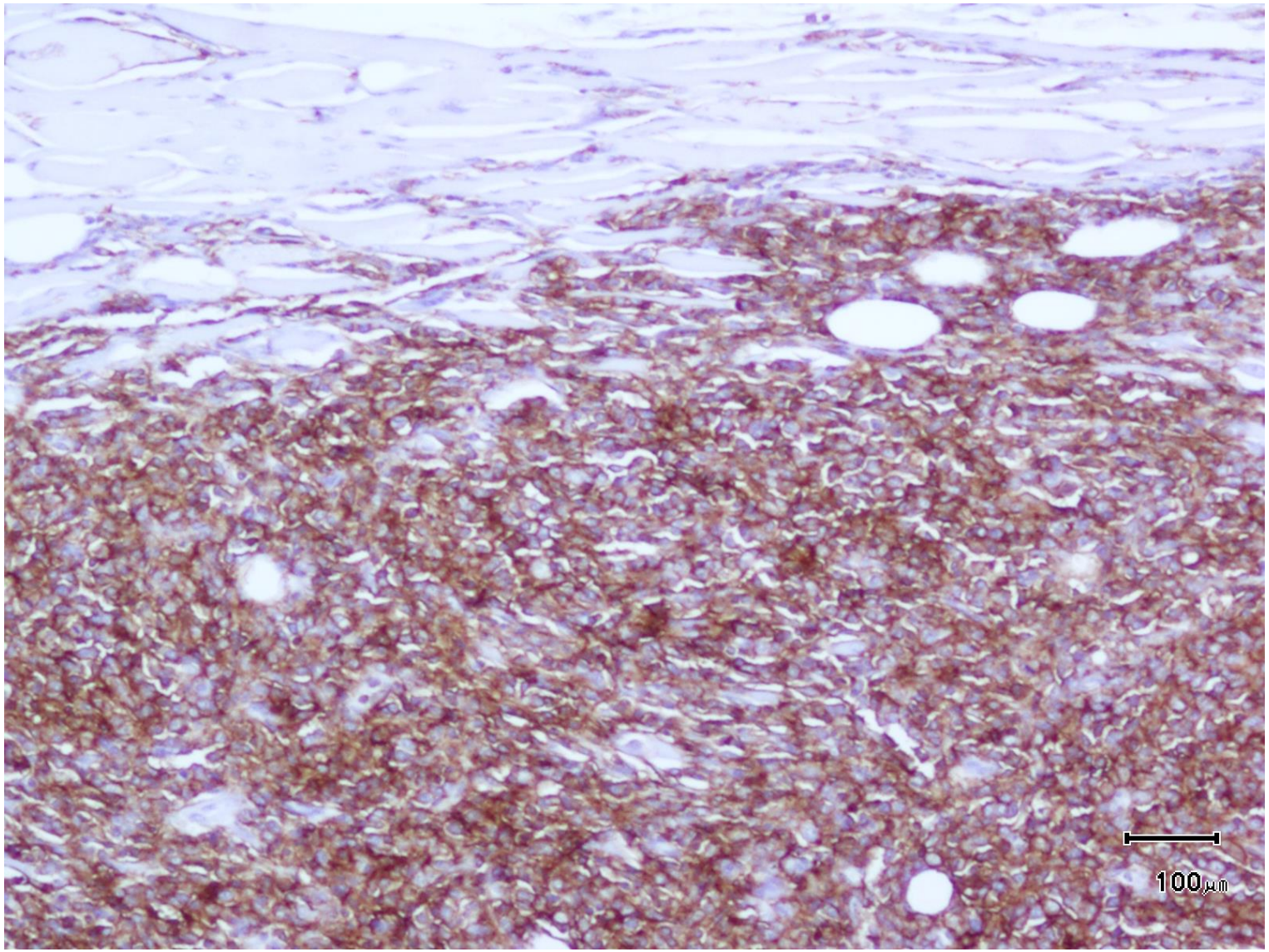
Liver with multiple nodular masses (arrows).



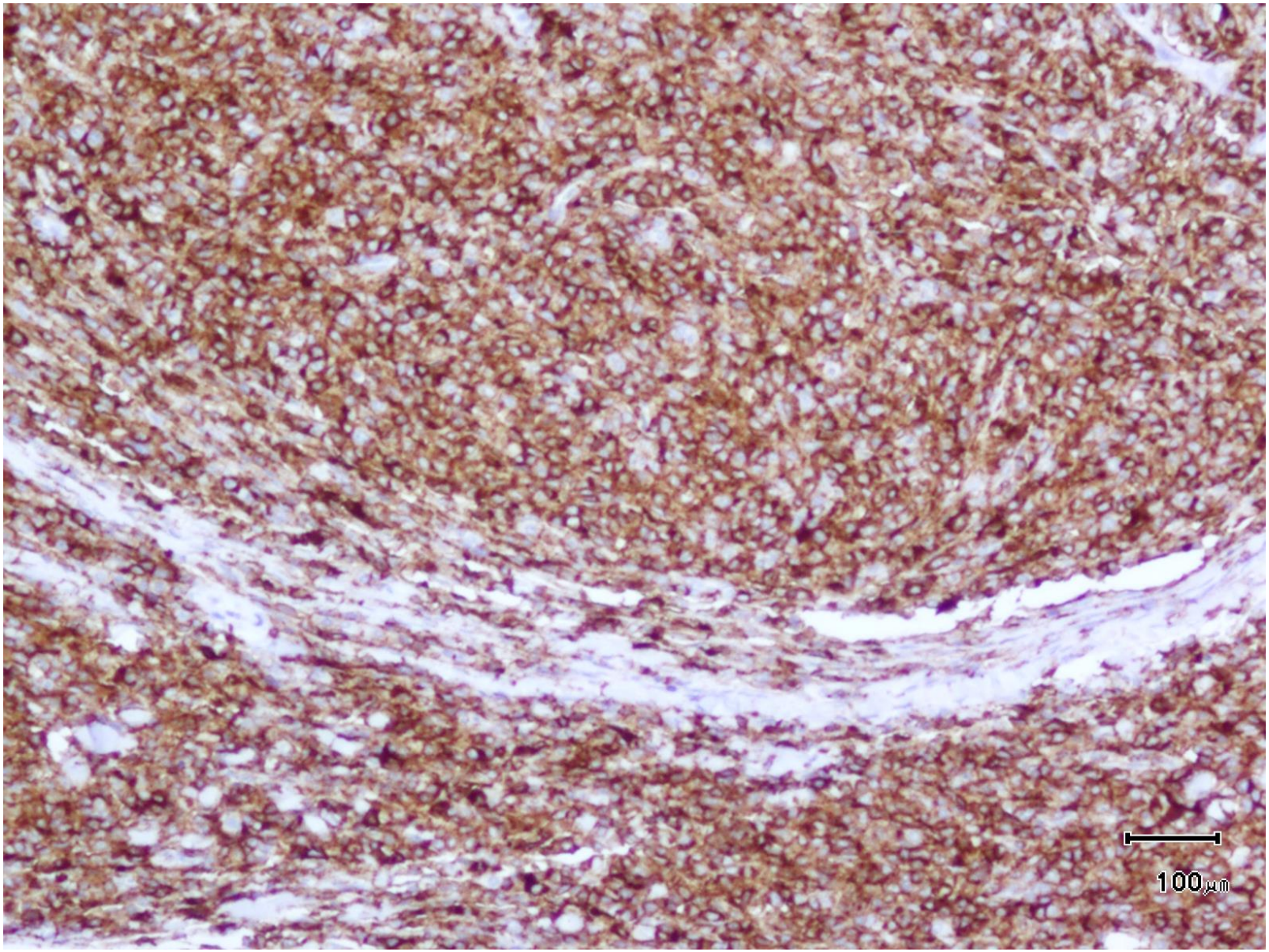
Vertebra body (T12) with a neoplastic infiltrative mass (arrows). Large arrow is the mass at the ventral aspect of the vertebra. Haematoxylin-eosin stain.



Neoplastic cells consistent with a histiocytic sarcoma. Haematoxylin-eosin stain.



Positive staining of neoplastic cells for CD18. Immunohistochemistry – DAB.



Neoplastic cells exhibit staining for MHC class II. Immunohistochemistry – DAB.

Histiocytic sarcoma - treatment

Localised:

- Surgery
- Radiotherapy
- ? Chemotherapy

Disseminated:

- ? Chemotherapy

Localised HS & radiotherapy

6 flat-coated retrievers with histologically confirmed histiocytic sarcomas of the limb
Five dogs were male and 1, female, ages ranged from 7 – 11yrs (mean 8.4).

Tumours were sited at

- Carpus, 1,
- Proximal forelimb 3,
- Stifle, 1
- Thigh 1

All tumours were of substantial mass, approx, mean 8 x 10 cm
All were associated with moderate to severe lameness.

Treatment
Palliative RT with 4 x 8.5 Gy fractions of megavoltage radiation (4MV) at 7 day intervals.

Localised HS & Radiotherapy

All tumours showed a marked and rapid response to radiation, by week 4 (day 28),

2 had completely regressed and the others had only residual swelling at the primary site.

All dogs showed a significant improvement in lameness with 4 / 6 becoming sound by the end of treatment.

Three of 6 dogs had metastatic disease at presentation and all eventually succumbed to disseminated disease, (mean survival 7 months, range 0 – 24m).

HS & Chemotherapy??

- A few reports of responses to doxorubicin, liposomal doxorubicin, paclitaxel
- Immunotherapy with human cytotoxic T-cell line (TALL-104)
- Lomustine

HS & Lomustine

Skorupski et al, JVIM 2007: 21: 212 - 216

- 59 dogs (56 dogs gross disease)
  - 6 dogs solitary lesions, 22 dogs 2 sites, 31 dogs 3 + sites.
- Lomustine 70mg/m^2 q 3 - 4 weeks
- Response:

  5 - CR, 21- PR (46% RR), 10 SD, 20 PD
- Survival

  MST responders = 172 days, non responders = 60 days, MST all dogs = 106 days (2 - 884 days)

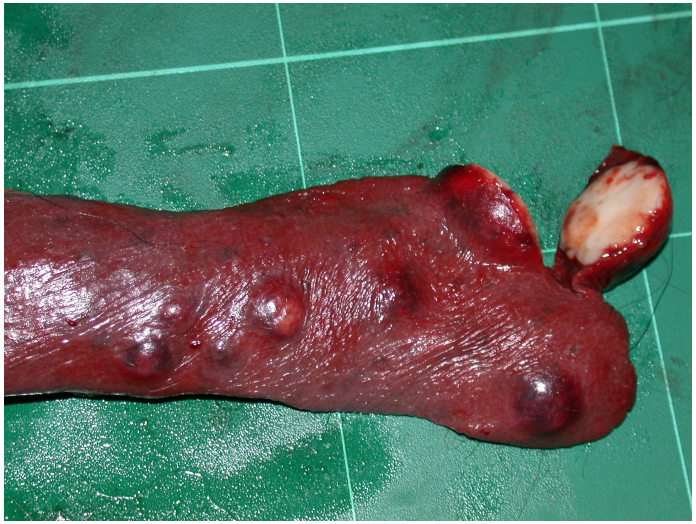
HS and Lomustine

Poor prognostic indicators

- thrombocytopenia
- hypoalbuminaemia
- splenic involvement

? Variation in response by

- anatomic presentation
- breed??

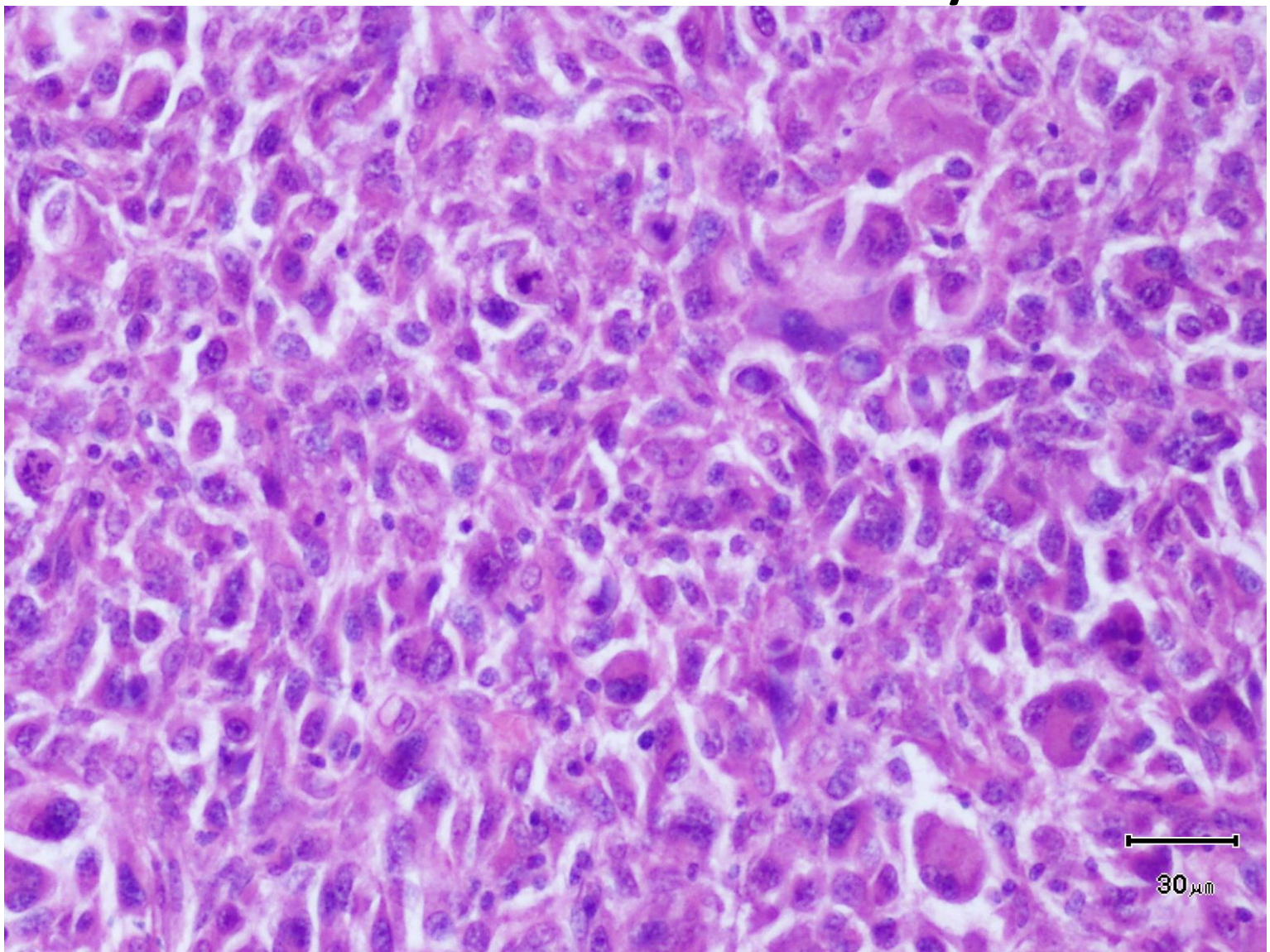


We still have much to learn about histiocytic sarcoma!

Future directions:

- Research
- Genetics
- Working with the breed

Could the micro-environment of the tumour hold the key?



“Evaluation of the microenvironment and immune function in Histiocytic sarcoma, a tumour of dendritic cells”

Drs Dobson, Blacklaws & Marcinowska

OPEN ACCESS Freely available online

PLOS | ONE

Gene Expression Profiling of Histiocytic Sarcomas in a Canine Model: The Predisposed Flatcoated Retriever Dog

Kim M. Boerkamp[1]*, Marieke van der Kooij[1☯], Frank G. van Steenbeek[1☯], Monique E. van Wolferen[1], Marian J. A. Groot Koerkamp[2], Dik van Leenen[2], Guy C. M. Grinwis[3], Louis C. Penning[1], Erik A. C. Wiemer[4], Gerard R. Rutteman[1]

1 Faculty of Veterinary Medicine, Department of Clinical Sciences Companion Animals, Utrecht University, Utrecht, The Netherlands, **2** Molecular Cancer Research, University Medical Centre Utrecht, Utrecht, The Netherlands, **3** Faculty of Veterinary Medicine, Department of Pathobiology, Utrecht University, Utrecht, The Netherlands, **4** Dept. of Medical Oncology, Daniel den Hoed Cancer Center, Erasmus University Medical Center, Rotterdam, The Netherlands

Abstract

Background: The determination of altered expression of genes in specific tumor types and their effect upon cellular processes may create insight in tumorigenesis and help to design better treatments. The Flatcoated retriever is a dog breed with an exceptionally high incidence of histiocytic sarcomas. The breed develops two distinct entities of histiocytic neoplasia, a soft tissue form and a visceral form. Gene expression studies of these tumors have value for comparable human diseases such as histiocytic/dendritic cell sarcoma for which knowledge is difficult to accrue due to their rare occurrence. In addition, such studies may help in the search for genetic aberrations underlying the genetic predisposition in this dog breed.

Methods: Microarray analysis and pathway analyses were performed on fresh-frozen tissues obtained from Flatcoated retrievers with localized, soft tissue histiocytic sarcomas (STHS) and disseminated, visceral histiocytic sarcomas (VHS) and on normal canine spleens from various breeds. Expression differences of nine genes were validated with quantitative real-time PCR (qPCR) analyses.

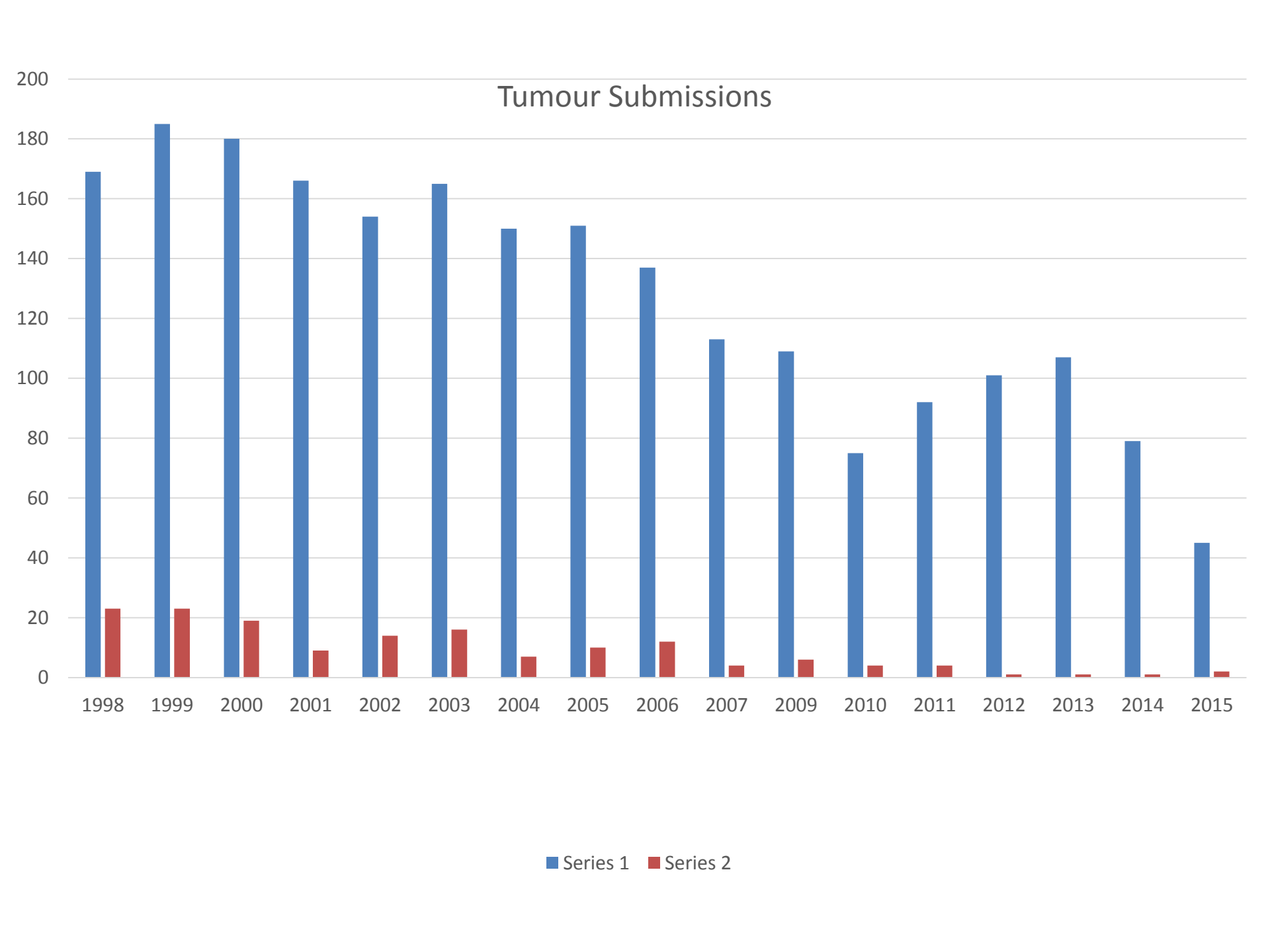
Results: QPCR analyses identified the significantly altered expression of nine genes; *PPBP, SpiC, VCAM1, ENPEP, ITGAD* (down-regulated), and *GTSF1, Col3a1, CD90* and *LUM* (up-regulated) in the comparison of both the soft tissue and the visceral form with healthy spleen. DAVID pathway analyses revealed 24 pathways that were significantly involved in the development of HS in general, most of which were involved in the DNA repair and replication process.

Conclusions: This study identified altered expression of nine genes not yet implicated in histiocytic sarcoma manifestations in the dog nor in comparable human histiocytic/dendritic sarcomas. Exploration of the downside effect of canine inbreeding strategies for the study of similar sarcomas in humans might also lead to the identification of genes related to these rare malignancies in the human.

Dr Maja Arendt, Uppsala & Cambridge
Swedish Flat-coated Retriever Breed Society

Tumour survey 1990 - 2015

- 2178 Submissions
- 156 external path reports or FNA's
- Archive of > 2,000 tissue samples


Tumour Submissions
200
180
160
140
120
100
80
60
40
20
0
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2009
2010
2011
2012
2013
2014
2015
Series 1
Series 2

Cause of Death Register

Cambridge Flatcoat Retriever Cause of Death Survey

This is a website to record data about the causes of deaths in Flatcoated Retrievers. It should take less than 5 minutes to complete. There are 7 screens including this one. You will be asked for the KC registration number of the dog, dates of birth and death, ages of the sire and dam at death (if deceased) and information about the cause of death. You may wish to look these up before starting.

We may not have anticipated every circumstance and so please enter any information you think might be relevant (such as more than one cause of death, or other fact that might not be caught by the categories listed) in the comments field on the 'confirm data' screen (screen 6).

Thank you for your help.

next

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- Launched 2013
- March 2016 : 412 dogs entered

- 195 bitches: 140 neutered
- 217 dogs: 106 neutered
- 26 Liver, remainder Black

Cause	Number affected N	%	comment
Endocrine disease	0		
Gastrointestinal disease	10		5 GDV
Haematological	10		Haemolytic anaemia, 3
Heart disease	16		
Kidney disease	19		Not specified
Liver disease	4		
Musculo-skeletal	4		
Neurological	6		
Old Age	30		
Other	19		Suspected haemorrhage, 3
Respiratory disease	3		1 x laryngeal paralysis
Trauma / accident	5		RTA most common
Tumour / cancer related	268	65%	See separate table
Unknown	17		Quite a few “sudden death”
total			

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