

Published Veterinary Times Volume 46 No 27 p14-18

The Flatcoated Retriever Tumour Survey, 25 years on

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Shortly after I (JD) came to Cambridge as Lecturer in Veterinary Oncology, we were approached by the late Sheila Godbolt and Martin Roe (MRCVS), who were concerned that a large number of Flatcoated retrievers were dying from Cancer. Initial attempts to determine the type(s) of tumour and the numbers of dogs affected were met with anecdotal reports of dogs with "liver cancer" or "stomach cancer" and it soon became apparent that a histological approach would be necessary to define the problem, if one existed. In March 1990 the "Tumour Survey" was started, veterinarians in general practice from across the UK were invited to submit tissues suspected of being neoplastic collected from Flatcoated retrievers, either by biopsy, excision or at Post Mortem examination, to the Department of Veterinary Medicine, University of Cambridge. Specimens were submitted in formalin and processed routinely to obtain haematoxylin and eosin (H & E) stained sections which were examined and reported by colleagues in pathology. The service was free to submitting veterinary surgeons and owners, with the basic costs of processing covered by owners & breeders of Flatcoated retrievers, who raised funds to support this work.

As word spread through the breed about the Tumour Survey, the number of submissions rose year on year and by 1998 we had received over 1000 samples, from 782 dogs. One hundred and sixty five samples (16%) were not neoplastic at all (cysts, skin tags, etc.), the remainder were evenly split between benign (447 lesions, 44%) and malignant (411 lesions, 40%). Perhaps not surprisingly canine cutaneous histiocytoma (CCH) was the most common of the benign submissions, accounting for 48% of benign tumours and 25% of all tumour submissions. However, the striking finding, which we reported in 2000 (Morris et al.) was that soft tissue sarcomas accounted for over 55% of the malignant tumours, and 25% of all tumour submissions. Many of these sarcomas were notable for being very poorly

differentiated and unclassifiable on standard H & E sections, being variably described as poorly differentiated (n = 73) spindle cell (n = 37), round cell (n = 20), histiocytic (n = 8) or giant cell (n = 3) (Figures 1 & 2). Although the predominant cell type varied between tumours, various cell types were often observed within different areas of the same tumour, many tumours had an inflammatory infiltrate, later shown to consist predominantly of T lymphocytes. Mitotic activity was common with between 3 – 5 mitoses per high power field. The majority of these tumours had arisen deep in the soft tissue / fascia of the limbs (49 fore limb, 34 hind), particularly affecting the upper forelimb between elbow and shoulder (Figure 3). Some visceral sites were also recorded. The mean age of dogs affected by these undifferentiated sarcomas was 8 years (range 0 – 13), 78 were female and 60 male.

Over the following years we continued to accrue samples from Flatcoated retrievers through the Tumour Survey, averaging at times up to 150 – 180 samples per year. The pattern of submissions with regards to tumour type has not changed substantially from that reported in 2000, and through this and other publications the tendency for Flatcoated retrievers to develop this particularly aggressive form of sarcoma has become well established. The Tumour Survey has provided an archive of tumours from Flatcoated retrievers which has proved a valuable resource for further research directed at determining the nature of these sarcomas. In 2002 we described the immunological and histopathologic features of 14 such tumours (Morris et al., 2002). The majority of tumours showed 100% positivity for vimentin, > 70% positive staining for MHCII with variable but < 50% staining for actin and desmin. We concluded that these undifferentiated sarcomas belong to a spectrum of tumours with varying proportions of characteristic cell types and morphological features, some of which fitted the diagnostic criteria for malignant fibrous histiocytoma (*A malignant pleocellular neoplasm, presumably arising from primitive mesenchymal cells, showing evidence of a fibroblastic / myofibroblastic phenotype*, Hendrick et al., 1998). Many of the tumours seemed to have a significant myofibroblast component and a moderate T cell infiltrate but the precise lineage remained uncertain.

Since this work published in 2002, more antibody reagents have become available to characterize cells, particularly those of the myeloid lineage, as a result of which terminology has changed. The term “Histiocytic sarcoma” (HS) has now been adopted to encompass two ends of a spectrum of malignant tumors previously referred to as malignant fibrous histiocytoma (MFH) and malignant histiocytosis (MH). The term localised HS has been proposed to describe solitary lesions and disseminated HS the multifocal form, previously

MH. The latter is highly breed specific especially in the Bernese Mountain Dog where it has been reported with a frequency of 25%. MH is also prevalent in rottweilers and other retrievers. In contrast to the multifocal, disseminated form of HS reported in these breeds, most forms of HS or HS-like tumours reported in Flatcoated retrievers have been solitary tumours arising in the deep musculature or fascia of limbs or in association with joints. However, we have documented an aggressive form of HS of the spleen in Flatcoated retrievers, and in these dogs the clinical presentation and findings were consistent with a haemophagocytic form of HS described by Moore and colleagues (Dobson et al., 2006)[Case study 1]. In 2008 the Tumour Survey provided information on 180 Flatcoated retrievers bearing HS-like lesions which showed that although the majority (101 lesions, 57%) were primary limb lesions, 47 dogs (26%) had visceral, mainly splenic lesions with no peripheral primary tumour (Figure 4).

HS have a range of histological appearances such that microscopically the diagnosis of HS can be complex. Histological findings include diffuse proliferation of neoplastic histiocytes, multinucleated histiocytic giant cells, spindle cells, anaplastic cells and in some cases presence of erythrophagocytic cells. Lymphocytic infiltrates in HS have also been reported. Immunohistochemical staining is an increasingly important technique to accurately identify the cell of origin in poorly differentiated tumours such as HS. Identification of histiocytes can be achieved with molecules involved in antigen presentation such as MHC class II molecules and the  $\beta 2$  integrins CD11d/CD18. On the basis of immunohistochemistry, HS is MHCII and CD18 positive, and the use of these markers has enabled HS to be differentiated from synovial sarcomas of the joint, and poorly differentiated sarcomas elsewhere in the body (Craig et al., 2002)

Also in 2008 we undertook a detailed histologic and immunohistologic review of 40 HS from the Survey, 20 limb tumours and 20 splenic tumours, and showed that 2 distinct phenotypic subtypes could be identified: a histiocytic subtype, most prevalent in the splenic tumours and a histiocytic-spindle-pleomorphic subtype, mainly seen in the limb tumours (Figures 5 & 6). Despite their variable morphology, all tumours expressed MHC class II and the leukocyte antigen CD18 but only those tumours in the spleen consistently expressed CD11d (Figures 7 & 8) (Constantino-Casas, et al., 2011). Since this time we have turned to molecular techniques using microRNA (miRNA) profiling as a means to gain a better understanding of soft tissue sarcomas and histiocytic sarcomas in particular. In a pilot study, containing 18 HS from FCR, the expression profile of localised and visceral HS did not lead to them forming

two different groups. Instead, at least three different HS groups could be identified, two of them having tumours from both locations. Since this was a pilot study, only the expression of 20 mature miRNAs were studied and it is possible that miRNAs that distinguish the two HS tumour types were not included in the study. Recently a group from the Netherlands have shown variation in gene expression between localised and visceral HS (Boerkamp et al., 2014).

### **Biological Behaviour, Management & Prognosis**

Sadly, the prognosis for dogs with histiocytic sarcoma is poor. Those dogs presenting with the disseminated form of the disease are often very sick at the time of diagnosis due to metabolic or haematological complications. The bone marrow is often affected and some forms of the disease are associated with haemophagocytosis (Dobson et al., 2006). Although localised histiocytic sarcomas may be managed initially by surgical excision, the site and extent of the lesion often precludes surgery short of amputation. Primary tumours can be sensitive to radiation, which offers an alternative to amputation for pain relief (Dobson, 2007). However, in the Flatcoated retriever, there is a high (70 - 90%) rate of distant metastasis, particularly to viscera including liver, spleen and kidneys (Figure 9). We have also documented unusual patterns of metastasis with diffuse infiltration of the leptomeninges leading to an acute neurological deterioration (Marcinowska et al, 2014) [Case Study 2].

There is some indication that the anticancer drug lomustine (CCNU) may play an adjuvant role in the management of localised HS: in a small study of 16 dogs treated with aggressive local therapy and adjuvant lomustine chemotherapy, median survival for all dogs of 568 days was reported. However, 2 dogs had local recurrence and 8 dogs developed metastatic disease and in these cases the median time to relapse was 201 days (Skorupski et al., 2009). The response rate to lomustine in 56 dogs with gross disease was reported to be 46% (overall response) but with a median survival of 106 days (Skorupski et al., 2007). More recently a combination chemotherapy protocol with the addition of doxorubicin to lomustine (+/- cyclophosphamide) has shown efficacy in some dogs with histiocytic sarcoma (Cannon et al, 2015) and liposomal clodronate has also been reported to have some efficacy in treatment (Hafeman et al, 2010). Many dogs with disseminated histiocytic sarcoma are euthanased at the time of diagnosis due to the widespread nature of the lesions and associated morbidity. Disseminated HS is generally considered to be poorly responsive to therapy. It remains to be seen whether more targeted therapies might play a role in the management of histiocytic disease in the future, first we need to identify potential targets.

## Cohort Study

The Tumour Survey provided valuable information on the type of tumour affecting the breed but did not really provide an indication of the prevalence of the problem within the breed. In 1994, we recruited 174 healthy dogs aged 2 – 7 to a “Health Study” and these were followed by an annual health census until death (Dobson et al., 2009). Seventy-two dogs (42%) died from confirmed neoplasia. Twenty dogs (11.6%) died of unconfirmed tumours and 61 (35%) died from non-neoplastic conditions. The cause of death was unknown for 19 dogs and 2 dogs were lost to follow up. Soft tissue sarcoma (especially histiocytic sarcoma) was the predominant cancer type, affecting 32 dogs (44% of neoplasms). Six dogs died with malignant melanoma and three with lymphoma. Median age at death was 9 years for dogs with tumours and 12 for non-neoplastic fatalities. The results confirm that soft tissue sarcoma, particularly histiocytic sarcoma is a major cause of mortality in the breed, age of onset for sarcoma shows a major peak at 8 and a minor one at 11 years, with an average age of onset at 8.13 years.

Examination of the pedigrees of affected dogs in the Health Study, plus a further 170 affected dogs identified through the ongoing Tumour Survey shows they all share 6 ancestors (3 males and 3 females) 4 to 9 generations in the past, suggesting a close relationship between all affected FCR. Examination of affectation status within sibships strongly suggests that the disease is familial (Figure 10). (Aguirre-Hernandez J. et al., 2005) The molecular genetics of histiocytic sarcoma has been investigated by molecular cytogenetic profiling (Hedan et al., 2011) and genome wide association studies (Shearin et al., 2012). Using genome wide array comparative genomic hybridization; copy number aberrations (CNAs) were assessed in 146 histiocytic sarcomas, 101 from Bernese mountain dogs (68 from USA & 33 from France) and 45 from Flatcoated retrievers (all from USA) (Hedan et al., 2011). Numerous CNAs were found, both gains and losses, throughout the genome, almost all of which were shared between the two breeds, suggesting that they are more associated with the cancer phenotype than with breed and a subset suggested involvement of known cancer associated genes including deletions of the tumor suppressor genes *CDKN2A/B*, *RB1* and *PTEN*. Interestingly dysregulation of *CDKN2* has also been associated with susceptibility to histiocytic sarcoma in Bernese mountain dogs by genome wide association study (GWAS) (Shearin et al., 2012).

In some dog breeds and in some other species, increased disease risk including increased risk of particular cancers, is associated with the presence or absence of particular major

histocompatibility complex (MHC) alleles. We have also investigated whether there are any associations between a diagnosis of histiocytic sarcoma and MHC II allotype in 40 affected Flatcoated retrievers compared to 40 control Flatcoated retrievers. In this population the MHC class II diversity was very restricted, but there was no significant difference between affected and controls, making it unlikely that MHC alleles have any causative relationship with the high prevalence of histiocytic sarcoma in the breed.

### **For the Future**

Since its beginnings, we have received 2,975 submissions to the Tumour Survey. Many of these submissions had more than one sample for processing, making the total number of samples well over 3,000. These, along with the information collected have been invaluable in understanding the high incidence of histiocytic sarcoma within the breed and will provide a valuable archive of tissue for future studies, specific details of these tumours will be published separately.

After 25 years of accruing data and samples, we believe that the tumour survey has served its purpose and run its course. The number of samples submitted has declined year on year for the past 5 years, and Tess Hoather, the stalwart of running the Survey has recently retired. The Tumour Survey will close at the end of 2015 and we will no longer offer the free histopathology service for the Breed. However, our interest in Flatcoated retrievers and other breeds affected with histiocytic sarcoma will not end. In 2013 in conjunction with the Flatcoated Retriever Society's Breed Health Sub-Committee, we set up a website data-base to monitor the "health" of the breed by recording the cause of death when dogs die. We believe that this is an innovative and effective way to monitor the health of the breed that may shed light on new or emerging problems and show trends in health issues. In the event of a FCR death we very much hope readers will encourage owners to help the Breed by visiting the Flatcoated Retriever Breed Website, clicking on Health, then follow the links to the Cause of Death Register and completing the short questionnaire.

### **Future studies**

The microenvironment of the tumour along with the immune system has an important role in both the development and progression of cancer. On the one hand the immune system can eradicate emerging malignant cells, but on the other by influencing the tumour

microenvironment, it can promote the growth, invasion and metastasis of malignant cells. In recent years the tumour microenvironment and the presence of infiltrating immune cells (T cells and macrophages) has become of increasing importance to our understanding of the relationship between cancer and the immune system. Of particular interest is the recognition of regulatory T cells within tumours that appear to down regulate the immune system and are associated with a poorer outcome in many human tumours. These cells may offer a target for future cancer management strategies so their role in cancer progression is important to understand. We have demonstrated that many of the T cells infiltrating histiocytic sarcomas are indeed regulatory T cells (Marcinowska et al., 2014)

Although the precise cellular origin of histiocytic sarcoma is unknown, the immunophenotype is suggestive of a myeloid dendritic antigen presenting cell (APC) lineage. It is interesting that this prominent T cell infiltrate should be present in a tumour comprising cells that modulate the immune response, dendritic cells. It is known that dendritic cells play a pivotal role in determining immune-tolerance versus immunity. Thus a key step in better understanding the relationship between the tumour, its microenvironment and the immune system is essential to understanding how the tumour influences immune function. The finding that a significant proportion of tumour infiltrating T cells expressed FOXP3, suggesting them to be regulatory T cells, raises interesting questions of cause and effect which we aim to address in future studies.

Our team at QVSH CTU (Figure 11) maintains a strong interest in the breed and in HS in general. Dr Sarah Mason has a particular interest in the medical and multi-modal therapy of HS, and Dr Marcinowska continues her research into HS tumour microenvironment. We would therefore very much like to hear of Flatcoated retrievers affected with histiocytic sarcoma. The QVSH cancer therapy team are always happy to see patients diagnosed with HS and to advise on the treatment options which can be adapted for individual patients. We are also pleased to advise on any aspect of diagnosis and case management, and would welcome the opportunity to receive tissue collected for diagnostic purposes from such cases.

### **Acknowledgements**

The Trustees of the Flatcoated Retriever Tumour Survey, and all those who have raised funds to support this work

Madeline Fordham & all the staff in the Histopathology laboratory who have processed the samples

Dr David Bostock and the many Pathologists & Residents who have reported for the Tumour Survey over the years

Dr David Sargan & Dr Jesus Aguirre-Hernandez for contribution to molecular & genetic based studies.



## References

Aguirre\_hernandez J., Dobson JM., Hoather T. Sargan D. Inheritance of histiocytic sarcomas in flat-coated retrievers. BSAVA Congress 2005 Scientific Proceedings p 634

Boerkamp KM, van Steenbeek FG, Penning LC et al, the two main forma of histiocytic sarcoma in the predisposed Flatcoated retriever dog display variation in gene expression. Plos one 2014, 9 (6) e98258

Cannon C., Borgatti A, Henson M., Husbands B. Evaluation of a combination chemotherapy protocol including lomustine and doxorubicin in canine histiocytic sarcoma. Journal of Small Animal Practice 56(7): 425 – 9, 2015

Constantino-Casas F., Mayhew D., Hoather T.M. Dobson J.M. The presentation and classification of histiocytic sarcomas in the flat-coated retriever. Veterinary Pathology 2011 48: 764-771,

Craig LE, Julian ME, Ferracone JD: The diagnosis and prognosis of synovial tumors in dogs: 35 cases. Veterinary Pathology 39:66-73,2002.

Dobson JM Radiation response of appendicular histiocytic sarcoma in flat-coated retrievers. ESVONC Spring meeting, Cambridge, March 2007

Dobson J.M. Villiers E., Roulois A., Gould S., Mellor P., Hoather T., Watson P. Histiocytic sarcoma of the spleen in flat-coated retrievers presenting with regenerative anaemia and hypoproteinaemia. Veterinary Record 158 (24) 825 – 829, 2006

Dobson J.M., Hoather T., McKinley T.J. & Wood J. Mortality in a Cohort of Flat-coated Retrievers in the UK. Veterinary and Comparative Oncology, 7 (2) : 115 – 121, 2009

Hafeman S., London C., Elmslie R., Dow S. Evaluation of liposomal clodronate for treatment of malignant histiocytosis in dogs. Cancer Immunol Immunother 59 (3): 441 – 52, 2010

Hedan B, Thomas R, Mostinger-Reif A, Abadie J, Andre C, Cullen J, Breen M. Molecular cytogenetic characterization of canine histiocytic sarcoma: a spontaneous model for human histiocytic cancer identifies deletion of tumor suppressor genes and highlights influence of genetic backgrounds on tumor behavior BMC Cancer 11: 201, 2011.

Hendrick MJ, Mahaffey EA, Moore FM, Vos JH, Walder EJ. Histological classification of mesenchymal tumors of skin and soft tissues of domestic animals. World Health Organisation International Classification of Tumours of Domestic Animals, Second Series Volume II, Armed forces Institute of Pathology, American Registry of Pathology, Washington DC. 1998

Marcinowska A; Constantino-Casas F; Dobson JM. Histiocytic sarcoma in a flat coated retriever with central and peripheral nervous system sarcomatosis. Veterinary Record Case Reports 2014;2:1.

Marcinowska A, Hoather T, Williams T, Dobson J, Constantino-Casas F Assessment of infiltrating lymphocytes in histiocytic sarcoma of Flat-coated retriever: a comparison of different locations. ESVONC Congress 2014.

Morris JS., Bostock DE., McInnes EF., Hoather TM., Dobson JM. A histopathological survey of neoplasms in flat-coated retrievers: 1990 – 1998. *Veterinary Record* 147: 291 – 295, 2000.

Morris JS., McInnes EF., Bostock DE., Hoather TM., Dobson JM. Immunohistochemical and Histopathologic features of 14 malignant fibrous histiocytomas from flat-coated retrievers. *Vet Pathol* 39: 473 - 479, 2002.

Mellanby RJ., Holloway A., Chantrey J., Herrtage ME & Dobson JM. Immune-mediated haemolytic anaemia associated with a sarcoma in a flat-coated retriever [Case Report] *Journal of Small Animal Practice* 45: 21 - 24, 2004

Shearin AL, Hedan B, Cadieu E, Erich SA, Schmidt EV, Faden DL, Cullen J, Abadie J, Kwon EM, Grone A, Devauchelle P, Rimbault M, Karyadi DM, Lynch M, Galibert F, Breen M, Rutteman GR, Andre C, Parker HG, Ostrander EA. The MTAP-CDKN2A locus confers susceptibility to a naturally occurring canine cancer. *Cancer Epidemiology Biomarkers & Prevention* 21(7):1019-1027, 2012.

Skorupski KA, Clifford CA, Paoloni MC, Lara-Garcia A, Barber L, Kent MS, LeBlanc AK, Sabhlok A, Mauldin EA, Shofer FS, Couto CG, Sørensen KU: CCNU for the treatment of dogs with histiocytic sarcoma. *Journal of Veterinary Internal Medicine* 21:121–126, 2007.

Skorupski KA, Rodriguez CO, Krick EL, Clifford CA, Ward R, Kent MS. Long-term survival in dogs with localized histiocytic sarcoma treated with CCNU as an adjuvant to local therapy. *Veterinary and Comparative Oncology* 2009 7(2) 139 – 44.