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## **Other studies**

During my Phd I was involved in several studies out of my main program, which will be briefly described below.

M. Novacco, S. Comazzi, L. Marconato, **M. Cozzi**, D. Stefanello, L. Aresu and V. Martini. **Prognostic factors in canine acute leukaemias: a retrospective study.** *Vet Comp Oncol.* 2015 Dec;14(4):409-416.

Canine acute leukaemias (ALs) have a poor prognosis, with reported survival times (ST) of only a few weeks or months. Also, clinical studies assessing prognostic factors are lacking. This study aims to retrospectively assess variables that predict ST in dogs with AL, and to identify correlations between outcome and therapeutic protocols. Diagnosis and sub-classification into AL subtypes was made based on haematological findings, morphological assessment and flow cytometric immunophenotyping. Clinical-pathological features of AL subtypes at presentation concurred with those described in the literature. A normal neutrophil count at presentation significantly prolonged ST ( $P = 0.027$ ). Additionally, there was a trend for anaemic dogs to have shorter survival compared with those without anaemia, and the incorporation of cytosine in the chemotherapy protocol produced a moderate but not significant increase in median ST for dogs with AL. Further prospective studies with standardized treatments are needed to confirm and improve our results.

[\*\*Martini V, Bernardi S, Marelli P, Cozzi M, Comazzi S. Flow cytometry for feline lymphoma: a retrospective study regarding pre-analytical factors possibly affecting the quality of samples.\*\*](#) J Feline Med Surg. 2017 Jul 1:1098612X17717175. doi: 10.1177/1098612X17717175.

**Objectives** Flow cytometry (FC) is becoming increasingly popular among veterinary oncologists for the diagnosis of lymphoma or leukaemia. It is accurate, fast and minimally invasive. Several studies on FC have been carried out in canine oncology and applied with great results, whereas there is limited knowledge and use of this technique in feline patients. This is mainly owing to the high prevalence of intra-abdominal lymphomas in this species and the associated difficulty in the diagnostic procedures needed to collect the sample. The purpose of the present study is to investigate whether any pre-analytical factor might affect the quality of suspected feline lymphoma samples for FC analysis. **Methods** Ninety-seven consecutive samples of suspected feline lymphoma were retrospectively selected from the authors' institution's FC

database. The referring veterinarians were contacted and interviewed about several different variables, including signalment, appearance of the lesion, features of the sampling procedure and the experience of veterinarians performing the sampling. Statistical analyses were performed to assess the possible influence of these variables on the cellularity of the samples and the likelihood of it being finally processed for FC. Results Sample cellularity is a major factor in the likelihood of the sample being processed. Moreover, sample cellularity was significantly influenced by the needle size, with 21 G needles providing the highest cellularity. Notably, the sample cellularity and the likelihood of being processed did not vary between peripheral and intra-abdominal lesions. Approximately half of the cats required pharmacological restraint. Side effects were reported in one case only (transient swelling after peripheral lymph node sampling). Conclusions and relevance FC can be safely applied to cases of suspected feline lymphomas, including intra-abdominal lesions. A 21 G needle should be preferred for sampling. This study provides the basis for the increased use of this minimally invasive, fast and cost-effective technique in feline medicine.

Stefano Comazzi, Stefano Marelli, **Marzia Cozzi**, Rita Rizzi, Riccardo Finotello, Joaquim Henriques, Josep Pastor, Frederique Ponce, Carla Rohrer-Bley, Barbara C Ruetgen, Erik Teske.

**Breed-associated risks for developing canine lymphoma differ among countries: an european canine lymphoma network study.** BMC Veterinary Research (under review)

Canine breeds may be considered good animal models to study genetic predisposition to cancer, as they represent genetic clusters. From epidemiologic and case collection studies it emerges that some breeds are prone to develop lymphoma or specific subtypes of lymphoma but available data are variable and geographically inconsistent. This study was born in the context of the European Canine Lymphoma Network with the aim to investigate the breed prevalence of canine lymphoma in different European countries and to define possible breed risk to develop lymphoma overall and/or different lymphoma subtypes.

A total of 1529 canine lymphoma cases and 55529 control cases, from 8 European countries/institutions were collected. Odds ratios to develop lymphoma vary among different countries but Doberman, Rottweiler, Boxer and Bernese mountain dogs showed a high

predisposition to develop lymphoma. In particular, Boxers were highly predisposed to T-cell lymphomas (either high- or low-grade) while Rottweilers had a high predisposition to B-cell lymphomas. Labradors were not predisposed to lymphoma overall but tended to develop mainly high-grade T-cell lymphomas. In contrast with previous studies outside of Europe, the European Golden retriever population did not show any predisposition to lymphoma overall or to specific subtypes such as T-zone lymphoma.

Breed-associated risks to develop lymphoma are different among European and American countries, mainly for Golden retrievers. Further studies to investigate possible genes involved in different predisposition between the two populations are needed to better elucidate this result.

Valeria Martini, Maverick Melega, Fulvio Riondato, Laura Marconato, **Marzia Cozzi**, Serena Bernardi, Stefano Comazzi, Luca Aresu. **Flow cytometric characterization of canine extranodal lymphomas: a retrospective study.** Journal of Veterinary Diagnostic Investigation (under review)

Flow cytometry (FC) is widely applied to characterize and stage nodal lymphomas in dogs, because it has a short turn-around time, requires a minimally invasive sampling procedure and allows a contemporary evaluation of neoplastic cells in the primary lesion and of blood and marrow involvement. It has also been applied to extranodal lymphomas in humans and cats, whereas its utility in extranodal forms is scarcely reported in dogs. The present study investigated advantages and limitations of FC in suspected extranodal lymphomas in dogs. Our results showed that the likelihood of obtaining a diagnostic FC sample was significantly lower for aspirates of extranodal lesions than for those of lymph nodes. However, no differences were obtained when comparing different extranodal lesion sites. We also described FC results of 39 cases of canine extranodal lymphomas. Neoplastic cells were commonly large-sized and easily identifiable on morphological FC scattergrams. Phenotypic aberrancies were frequently present, mainly in T-cell lymphomas. Thus, lymphoma cells were distinguishable from normal residual lymphocytes in >85% of cases, facilitating the quantification of blood and marrow involvement by FC. Based on our results, we support the inclusion of FC in the diagnostic workup of suspected extranodal lymphomas in dogs, despite the relevant percentage of non-diagnostic samples (32 out of 73, >40%), in conjunction with histopathology. This latter technique has a longer turn-around time but provides adjunctive

relevant information, including tissue-invasion and epitheliotropism. Histopathology is also mandatory in case of non-diagnostic FC samples.

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