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NEOPLASTIC DISEASE

World Health Organisation Classification of Lymphoid Tumours in Veterinary and Human Medicine: a Comparative Evaluation of Gastrointestinal Lymphomas in 61 Cats

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Summary

To diagnose and classify the various entities of lymphomas, the World Health Organisation (WHO) classification is applied in human as well as in veterinary medicine. We validated the concordance of these classification systems by having a veterinary and human pathologist evaluate gastrointestinal lymphoma tissue from 61 cats. In 59% of all cases, there was a match between their respective diagnoses of the lymphoma subtype. A complete consensus between the two evaluators was obtained for all samples with a diagnosis of diffuse large B-cell lymphoma, T-cell anaplastic large cell lymphoma and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue. A corresponding diagnosis was also made in the majority of samples with enteropathy associated T-cell lymphoma (EATL) type II, although this subtype in cats has similarities to the 'indolent T-cell lymphoproliferative disorder of the gastrointestinal tract', a provisional entity newly added to the revised human WHO classification in 2016. Very little consensus has been found with cases of EATL type I due to the fact that most did not meet all of the criteria of human EATL I. Hence, the human pathologist assigned them to the heterogeneous group of peripheral T-cell lymphomas (not otherwise specified). Consequently, concrete guidelines and advanced immunophenotyping based on the model of human medicine are essential to differentiate these challenging entities in veterinary medicine.

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Introduction

Lymphoma represents the most common malignancy in cats (MacVean *et al.*, 1978). The tumour cells develop from lymphoid cells of the immune system, thus any tissue or organ can be affected. Today, alimentary (synonyms: gastrointestinal, intestinal) lymphoma is considered to be the most common anatomical form of the tumour in cats (Louwerens *et al.*, 2005). Diagnostic steps in affected cats include ultrasonography of the abdomen and ultrasound-guided fine needle aspiration of the lesions, which often confirm the clinical suspicion of underlying lymphoma. However, for the identification of lymphoma subtypes, a thorough histopathological examination of a high-quality sample is mandatory.

In human medicine, a new histopathological classification system named the Revised European–American Classification of Lymphoid Neoplasms (REAL) was introduced in 1994 by the Human

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Medicine International Lymphoma Study Group. Morphology, immunophenotype, genetic abnormalities and biological behaviour of the disease were merged to differentiate between distinct lymphoid diseases (subtypes) in the heterogeneous group of human lymphomas. The REAL classification was superseded by the World Health Organisation (WHO) classification, first published in 2001 (Jaffe *et al.*, 2001), then updated in 2008 (Swerdlow *et al.*, 2008) and lately revised (Swerdlow *et al.*, 2016).

In human medicine, each lymphoma subtype of the WHO classification is considered to be a distinct disease entity. Therefore, each subtype must be assessed individually and treatment protocols for one lymphoma type are not necessarily favourable for other lymphomas, even if they originate from the same cell lineage (Jaffe *et al.*, 2008).

In veterinary medicine, the older National Cancer Institute Working Formulation (NCI-WF) and the updated Kiel classification system were superseded by the newer REAL/WHO classification (Valli *et al.*, 2002, 2011), complemented by the book *Veterinary Comparative Hematopathology* (Valli, 2007) and the revised WHO classification described in the text *Pathology of Domestic Animals* (Valli *et al.*, 2016). To date, few reports have described feline lymphoma subtypes categorized according to the WHO classification (Waly *et al.*, 2005; Pohlman *et al.*, 2009; Vezzali *et al.*, 2010; Moore *et al.*, 2012; Wolfesberger *et al.*, 2017).

The WHO classification systems for haemopoietic tumours used in veterinary medicine are very similar to those in human medicine. However, the concordance between these respective classification systems has not yet been examined. Therefore, one might wonder whether a human pathologist achieves the same results as a veterinary pathologist in evaluating feline lymphoma tissue. To answer this question, alimentary lymphoma samples from cats were assessed comparatively by a veterinary and human pathologist using their respective WHO classifications. In addition, we evaluated the correlation between the WHO subtypes and the different tumour locations in the gastrointestinal tract.

Materials and Methods

Tissue Specimens

Sixty-one samples of feline alimentary lymphoma were selected from the archive of the Institute of Pathology of the University of Veterinary Medicine, Vienna, Austria. The tumours originated from the stomach, small intestine, large intestine, mesenteric lymph nodes or combinations of these locations. From paraffin wax-embedded samples, sections (3–4 μm) were prepared and stained with haematoxylin and eosin (HE). Each slide was examined by a veterinary pathologist (AF) and a human pathologist (CB).

Immunohistochemistry

For identification of T-cell lineage (CD3) and B-cell lineage (CD79a), immunohistochemistry (IHC) was performed using a LabVision-Autostainer (Thermo Fisher Scientific, Fremont, California, USA) and for detection of CD30-positive cells IHC was performed on a Dako-Autostainer (Dako, Glostrup, Denmark). The biotin–streptavidin–peroxidase method was used.

For CD3 and CD79a labelling, slides were pretreated with heat in citrate buffer (pH 6.0) for 15 min for antigen unmasking. To decrease background, the slides were incubated in H_2O_2 block (Thermo Fisher Scientific) for 5 min and in Ultra V Block (Thermo Fisher Scientific) for another 10 min. A polyclonal rabbit anti-human antibody specific for CD3 (Dako; diluted 1 in 1,000) and a monoclonal mouse anti-human antibody specific for CD79a (Dako; diluted 1 in 300) were used as pan-T cell and pan-B cell markers, respectively. The samples were incubated with the primary antibodies for 30 min and with the secondary antibodies for 30 min (biotinylated goat anti-rabbit) or 15 min (biotinylated goat anti-mouse, both from Thermo Fisher Scientific), respectively. The binding reaction was ‘visualized’ by use of streptavidin peroxidase (Thermo Fisher Scientific; incubation for 20 min) and 3, 3′ diaminobenzidine (DAB; Large Volume DAB Plus Substrate System for 5 min; Thermo Fisher Scientific) as chromogen. Slides were counterstained with Mayer’s haematoxylin, dehydrated, placed into Neo Clear™ and mounted in Neo-Mount™ (both Merck, Darmstadt, Germany).

For reaction with an antibody to CD30, the slides were pretreated with Dako REAL™ Proteinase K solution for 10 min. A monoclonal mouse anti-human antibody specific for CD30 (clone HRS4 Labvision, Värmdö, Sweden; diluted in 1 in 50) was applied for 10–25 min, followed by the biotinylated secondary antibody (goat anti-mouse, Labvision) for 15 min. Subsequently, the slides were incubated in Dako REAL™ Peroxidase-blocking solution for 5 min. Streptavidin peroxidase (Dako REAL™ Detection System) was applied for 15 min and

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