Veterinary and Comparative Oncology

Original Article

DOI: 10.1111/j.1476-5829.2009.00201.x

Histopathologic classification of 171 cases of canine and feline non-Hodgkin lymphoma according to the WHO

E. Vezzali[1,2], A. L. Parodi[3], P. S. Marcato[1] and G. Bettini[1]

[1]Department of Veterinary Public Health and Animal Pathology, Pathological Anatomy Service Alma Mater Studiorum-University of Bologna, Italy
[2]Pathology Department, R.T.C.-Research Toxicology Center S.p.A., Via Tito Speri 12-14, I-00040 Pomezia, Rome, Italy
[3]Departement d'Anatomie Pathologique, Ecole Nationale Vétérinaire d'Alfort, France

Abstract

A retrospective collection of 171 lymphoid neoplasms (123 dogs and 48 cats) was classified according to the Revised European–American Lymphoma (REAL) classification, adopted in 2002 by the World Health Organization (WHO), to evaluate the WHO system for categorization of canine and feline neoplasms. Microscopic examination was performed after standard hematoxylin–eosin staining and immunohistochemical labelling for B (CD79a) or T (CD3) cell phenotypes. B-cell lymphomas were prevalent in dogs and T-cell lymphomas in cats. B-Large cell lymphoma (B-LCL) frequently showed plasmacytoid differentiation; notably, two canine plasma cell tumours (PCT) expressed both CD79 and CD3. There were difficulties in differentiating B-lymphoblastic lymphoma (B-LBL) from Burkitt-type lymphoma. Furthermore, intestinal T-cell lymphoma (ITCL) exhibited a huge morphologic variability. Finally, multicentric mature small and thymic T-cell lymphomas were diagnosed, although these categories are not codified by the WHO classification.

Keywords
cat, dog, immunohistochemistry, lymphoma, non-Hodgkin

Introduction

Lymphomas are the most common spontaneous haematopoietic neoplasms in dogs and cats. They also represent a good animal model for human non-Hodgkin, lymphoma, although in man there is a low proportion of high-grade lymphomas in comparison to pets.

As in human pathology, several systems of classification have been developed during the last decades for animal lymphoid tumours, based on morphological, immunophenotypic and, recently, genotypic criteria. All classification schemes have tried to correlate histotypes with biological behaviour, in order to achieve a reliable prognosis, to select efficient therapies and to give the pet owner comparative information.[1,2] The Kiel classification and its updated version introduced a key concept based on cyto-immunological criteria, and the National Cancer Institute Working Formulation (NCI-WF) simplified the lymphoma classification to high-and low-grade tumours. More recently, in 2002, World Health Organization (WHO) published the Revised European–American Lymphoma (REAL) classification of domestic animals.[3–11] This classification was adapted from human medicine, and it was enriched by other haematopoietic myeloid tumours under the guidance of the WHO panel of veterinary experts. The WHO classification system for animal lymphoma is a pragmatic list of haematopoietic neoplasms. It is drawn up regardless of the tumour origin (e.g. bone marrow or lymph nodes). Its main goal is to correlate each category of lymphoma to its own behaviour and degree of malignancy.[8,10,11]

Correspondence address:
E. Vezzali
Pathology Department
R.T.C.-Research Toxicology Center S.p.A.
Via Tito Speri 12-14
I-00040 Pomezia
Rome, Italy
e-mail: enrico.vezzali@tin.it

© 2009 Blackwell Publishing Ltd

The identification of B- or T-cell lineage is mandatory and generally based on the cell phenotype after hematoxylin–eosin staining and confirmed by immunophenotyping. In addition, topography of the tumour tissue must be considered.[8,10,12,13]

The aim of this study was to catalogue cases of canine and feline lymphoma by means of the WHO classification, looking for correlations with the Kiel classification and the WF. No consideration was given to other haemolymphopoietic entities. Again, we evaluated if there was a homogeneous representation of tumours within the different subtypes.

Materials and methods

A retrospective study was performed on more than 200 cases selected from 1984 to 2002, in which a diagnosis of lymphoma was originally obtained by routine histopathological examinations performed by P. S. M. and G. B. according to the NCI-WF. Tumours other than lymphoma (i.e. myeloproliferative malignancies) were not taken into consideration. The histological sections of lymphoma were re-evaluated with a multiobserver up-right bright-field microscope, according to the WHO by E. V. and A. L. P., reaching a consensus. For some cases, lymphoma was suspected on the basis of previous cytological examinations. Several cases were excluded from the review because of missing data, poor tissue details and inadequate samples. Samples that lacked immunoreactivity, because of excessive fixation and antigen denaturation, were excluded from the study based upon previous studies using alternative antibodies. All samples selected had been previously immunolabelled with antibodies such as Ki67, CD117, vimentin and pan-cytokeratin, so their fixation could be considered optimal. Finally, we selected 123 canine and 48 feline cases for this study.

For histopathology, samples were obtained mainly during necropsy and, in some cases, by biopsy and were fixed in 10% Carson neutral buffered formalin. Three to 5 μm sections obtained from blocks of paraffin wax were stained with hematoxylin–eosin (HE) and with immunohistochemical stains to demonstrate B- and T-cell lineage. Positive controls consisted of canine and feline normal lymph nodes, while negative controls were the same sections untreated with the chosen primary antibody. Tissue sections were hydrated utilizing xylenes and graded alcohol series. Successively, quenching of endogenous peroxidase activity was achieved by incubating the sections for 30 min in 0.3% hydrogen peroxide in methanol at room temperature. Antigen retrieval was achieved by applying two cycles of 5 min each in a microwave oven at 750 W to the sections, dipped in EDTA buffer (pH = 8.00). Primary antibodies were applied (except for negative controls), and incubated with buffer alone. Polyclonal rabbit anti-human CD79a clone HM57 (Dako Cytomation, CA, USA–code # M7051) diluted 1:50 in phosphate saline buffer (PBS) for B-cell and monoclonal mouse anti-human CD3 clone F.7.2.38 (Dako–code # M7254) diluted 1:75 in PBS for T-cell labelling. Incubation was completed overnight at 5°C for both antibodies. Streptavidin–biotin revelation commercial kit (Dako–code # K0690) was used and the reaction was developed for 7 min in a dark room at room temperature with diaminobenzidine (DAB). Counterstain was applied for 3 s by immersion in Papanicolau hematoxylin 1:2 in water as contrast.

Some cases required toluidin blue (TB), methyl-green pyronin (MGP), Gomori's silver impregnation and Masson's trichrome (MT) special staining, respectively, to assess more closely the nuclear and cytoplasmic detail, to emphasize the plasmacytoid pattern, and to identify reticulin and mature collagen fibres.

All slides were mounted with xylene-based mounting media DPX (Fluka Bio Chemika Sigma-Aldrich, MO, USA).

There were insufficient haematological and/or FIV–FeLV data available for cats and so this information was excluded.

Leukaemia alone was not considered in this study because of the lack of information regarding haematologic examinations of our cases.

The criteria used for identification of the various types and subtypes are as described in the WHO monograph on haematopoietic tumours of domestic animals[9] and are influenced by the recent literature covering this subject.[10] We did not use more recent editions of the human WHO classification because no official veterinary adaptation was available.

Results

The age of the animals varied from 2 to 13 (mean 7.9) years in dogs and from 1 to 16 (mean 9.3) years in cats. The breed distribution showed the prevalence of mixed-breed carriers (29/124), followed by the German Shepherd breed (26/124) in dogs. Cats were mainly represented by mixed 'European' breeds. The gender distribution in dogs was 66 entire males (M) or male castrates (C), 51 entire (F) or spayed (S) females and 6 cases that were unknown. In cats the gender distribution was 31 M or C and 17 F or S.

In Table 1, the categories of lymphoid neoplasms are quoted according to the WHO classification and to their equivalents in the WF. The main cellular pattern is given according to the Kiel classification. The results relating to the tumour types, divided by species, are summarized in Tables 2 (dogs) and 3 (cats).

As a general rule, histologically B-cell lymphomas appeared less pleomorphic than T-cell ones [except for lymphoblastic lymphomas (LBL)], with darker cytoplasmic staining and more eccentric nuclei (according to their possible plasmacytoid differentiation). Furthermore, T-cell lymphomas were more frequently associated with neoangiogenesis and inflammation.

Precursor cell neoplasms

In cases of *B- or T-acute lymphoblastic leukaemia* (*B- or T-ALL*) or their solid respective *B- or T-lymphoblastic lymphoma* (*B- or T-LBL*) (three dogs and one cat bearing precursor B-cell tumour; eight dogs bearing T-cell ones), densely stained cells of moderate size were present, with intermediate sized nuclei, finely distributed chromatin, almost indistinct small nucleoli and scant cytoplasm. They had a diffuse architecture, high mitotic rate and shallow nuclear indentations (in this case, subtyped as convoluted). A starry-sky appearance was evident at low magnification (Fig. 1A), even if, in canines, this type usually showed only few tingible body macrophages.

Mature cell neoplasms

B- or T-chronic lymphocytic leukaemia (*B-* or *T-CLL*) or their solid correlates *B-* or *T-small lymphocytic lymphoma* (*B-* or *T-SLL*) were identified in seven dogs, three with mature B-cell and four with mature T-cell disorders. This tumour type can be considered an accumulation, rather than a proliferation, of small cells with minimal cytoplasms, small round nuclei (1 to 1.5 red blood cell in diameter), large and dense chromocenters, no nucleoli and with very low mitotic rate. Two out of the four mature T-cell lymphomas we described were multicentric without leukaemic involvement.

B-cell lymphomas with nuclei slightly larger and more vesicular than B-SLL are called *B-cell lymphocytic lymphoma of intermediate type* (*LLI*) (seen in one dog). The same characteristics associated with a moderate quantity of densely stained eccentric cytoplasm are typical of *lymphoplasmacytic lymphoma* (*LPL*), which was observed in four dogs.

Large granular lymphocyte lymphoma (*LGL*) was observed in one cat. LGL lymphoma is a subtype of mature T-cell lymphoma, characterized by tumour cells bearing azurophilic cytoplasmic granules, which were readily evident in our cytological smears stained with May Grünwald–Giemsa, but imperceptible on histological slides.

Follicular neoplasms

All follicular lymphomas displayed a B-cell-type phenotype and were subtyped according to their follicular architecture, presence or absence of mantle cell cuffs and dominant population. Centrocytes are small lymphoid cells with clear cleaved cytoplasm, very pale nucleus with small densely stained chromocenters and no nucleoli; centroblasts are large lymphoid cells with a moderate amount of strongly basophilic cytoplasm, round or cleft nuclei, peripheral chromatin, multiple peripheral nucleoli and one to three prominent and peripheral chromocenters. According to the WHO classification, they were divided into three main groups:

(1) *mantle cell lymphoma* (*MCL*), with proliferation and coalescence of mantle cell cuffs, in two dogs;
(2) *follicle centre cell lymphoma* (*FCCL*), subdivided in to *grade I* (predominance of centrocytes), not observed; grade *II* (equal

Table 1. Number of observed cases according to the WHO classification, corresponding category in the Kiel classification and in the Working Formulation.

WHO classification			Working Formulation	Kiel classification	Number of dogs	Number of cats	Total number
B	Precursor	B-ALL/B-LBL	ALL, LBL	Lb	3	1	4
	Mature	B-CLL/B-SLL	CLL, SLL	Lc	3	0	3
		LLI	–	–	1	0	1
		LPL	SLLP	Pl	4	0	4
	Follicular	MCL	DSCCL	Cc	2	0	2
		FCCL-I	FSCCL	Cc	0	0	0
		FCCL-II	FMCL	Cc–Cb	2	1	3
		FCCL-III	FLCL	Cb	1	3	4
		NMZ	CLL, FSCCL, DSCCL, DMCL	Lc, Cc, Cb	3	0	3
		SMZ			0	0	0
		MALT-L			1	1	2
	Plasmacytic tumours	PCT Indolent	Extramedullary plasmacytoma	Extramedullary plasmacytic lymphoma	19	1	20
		PCT Anaplastic			1	1	2
		Myeloma	Multiple myeloma	Multiple myeloma	0	1	1
	B-LCL	DLBCL, DLBCCL	DMCL, DLCL, DLCCL	Cc, Cb	41	5	46
		LCIBL	LCIBL	Ib	13	3	16
		T-cell rich B-LCL	DMCL, DLCL	Cb	1	0	1
		Thymic B-LCL	DMCL, DLCL	Cb	1	0	1
	Burkitt-type, Burkitt-like		SNCCL	–	1	0	1
T	Precursor	T-ALL/T-LBL	ALL, LBL	Lb	8	0	8
	Mature	T-CLL/T-SLL	CLL, SLL	Lc	4	0	4
		LGL	–	–	0	1	1
		NK-CLL	–	–	0	0	0
	Cutaneous	CEL	MF/SS or DLCL, DMCL, DSCCL	MF/SS	2	0	2
		CNEL			4	0	4
			MF/SS-like or DLCL, DMCL, DSCCL	MF/SS-like			
	PTCL		DSCCL, DMCL, DLCL, DLCCL	–	3	11	14
	ATL	ACL	DSCCL, DMCL, DLCL, DLCCL	–	0	3	3
		AIL					
	ITCL		DSCCL, DMCL, DLCL, DLCCL, LCIBL	–	5	16	21
	ALCL		LCIBL	–	0	0	0
					123	48	171

B- or T-ALL, B- or T-acute lymphoblastic leukaemia; B- or T-LBL, B- or T-lymphoblastic lymphoma; B- or T-CLL, B- or T-chronic lymphocytic leukaemia; B- or T-SLL, B- or T-small lymphocytic lymphoma; LLI, B-cell lymphocytic lymphoma of intermediate type; LPL, lymphoplasmacytic lymphoma; LGL, large granular lymphocyte lymphoma or leukaemia; NK-CLL, NK-cell chronic lymphocytic leukaemia; MCL, mantle cell lymphoma; FCCL, follicle center cell lymphoma; MZL, marginal zone lymphoma; NMZ, nodal marginal zone lymphoma; SMZ, splenic marginal zone lymphoma; MALT-L, mucosa-associated lymphoid tissue lymphoma; PCT, plasmacytic tumours; B-LCL, large B-cell lymphoma; DLBCL or DLBCCL, diffuse large B-cell lymphoma, cleaved or not cleaved; LCIBL, large cell immunoblastic lymphoma; CEL, cutaneous epitheliotropic lymphoma; MF/SS, mycosis fungoides/Sezary syndrome; CNEL, cutaneous non-epitheliotropic lymphoma; PTCL, extranodal or peripheral T-cell lymphoma; AIL, angioimmunoblastic lymphoma (otherwise known as AILD, angioimmunoblastic lymphomatous disease); ATL, angiotropic lymphoma; ACL, angiocentric lymphoma; AIL, angioinvasive lymphoma; ITCL, intestinal T-cell lymphoma; ALCL, anaplastic large cell lymphoma; Lb, lymphoblastic; Lc, lymphocytic; Cc, centrocytic; Cb, centroblastic; Ib, immunoblastic; Pl, plasmacytic/plasmacytoid; F- or DSCCL, follicular or diffuse small cleaved cell lymphoma; F- or DMCL, follicular or diffuse mixed cell lymphoma; F- or DLCL, follicular or diffuse large cell lymphoma; DLCCL, diffuse large cleaved cell lymphoma; SLLP, small lymphocytic lymphoma plasmocitoid; SNCCL, small non-cleaved cell lymphoma; PCT, plasmacytoma.

Table 2. Canine lymphomas classified according to the WHO classification.

Number of dogs		WHO classification type
3	B-ALL/B-LBL	B-acute lymphoblastic leukaemia/B-lymphoblastic lymphoma
3	B-CLL/B-SLL	B-chronic lymphocytic leukaemia/B-small lymphocytic lymphoma
1	LLI	Lymphocytic lymphoma intermediate type
4	LPL	Lymphoplasmacytic lymphoma
2	MCL	Mantle cell lymphoma
2	FCCL-II	Follicular center cell lymphoma
1	FCCL-III	
3	NMZ	Nodal marginal zone lymphoma
1	MALT-L	Mucosa-associated lymphoid tissue lymphoma
19	PCT indolent	Plasmacytic tumour indolent type
1	PCT anaplastic	Plasmacytic tumour anaplastic type
41	DLBCL, DLBCCL	Diffuse large B-cell lymphoma, diffuse large B-cell cleaved lymphoma
13	LCIBL	Large cell immunoblastic lymphoma
1	T-cell rich B-LCL	T-cell rich B-Large Cell Lymphoma
1	Thymic B-LCL	Thymic B-Large Cell Lymphoma
1	Burkitt-type, Burkitt-like	Burkitt-type lymphoma, Burkitt-like lymphoma
8	T-ALL/T-LBL	T-acute lymphoblastic leukaemia/T-lymphoblastic lymphoma
4	T-CLL/T-SLL	T-chronic lymphocytic leukaemia/T-small lymphocytic lymphoma
2	CEL	Cutaneous epitheliotropic lymphoma
4	CNEL	Cutaneous non-epitheliotropic lymphoma
3	PTCL	Peripheral T-cell lymphoma
5	ITCL	Intestinal T-cell lymphoma

Table 3. Feline lymphomas classified according to the WHO classification.

Number of cats		WHO classification type
1	B-ALL/B-LBL	B-acute lymphoblastic leukaemia/B-lymphoblastic lymphoma
1	FCCL-II	Follicular center cell lymphoma
3	FCCL-III	
1	MALT-L	Mucosa-associated lymphoid tissue lymphoma
1	PCT indolent	Plasmacytic tumour indolent type
1	PCT anaplastic	Plasmacytic tumour anaplastic type
1	Myeloma	Multiple myeloma
5	DLBCL, DLBCCL	Diffuse large B-cell lymphoma, diffuse large B-cell cleaved lymphoma
3	LCIBL	Large cell immunoblastic lymphoma
1	LGL	Large granular lymphocytes lymphoma
11	PTCL	Peripheral T-cell lymphoma
3	ATL (ACL, AIL)	Angiotropic lymphoma (Angiocentric lymphoma, Angioinvasive lymphoma)
16	ITCL	Intestinal T-cell lymphoma

distribution of centrocytes and centroblasts, 6–15 per high power field), in three cases, two dogs and one cat; and grade *III* (predominance of centroblasts) in one dog;

(3) *marginal zone lymphoma* (*MZL*), of *nodal* (*NMZ*), *splenic* (*SMZ*) or *mucosal type* (*mucosa-associated lymphoid tissue lymphoma*, *MALT-L*), with abundant and relatively clear cytoplasm cells encircling the normal pre-existing follicles outside the mantle cell cuff, in five cases (three canine NMZ and two MALT-L in one dog and one cat).

Plasmacytic neoplasms

There were 23 *Plasmacytic tumours* (*PCT*) (20 dogs and 3 cats). These tumours were characterized by cells of variable size, with deeply stained and eccentric cytoplasm, with irregular evident Golgi apparatus (clear *juxta*-nuclear bean-shape area), frequently double oval nuclei and small or absent nucleoli. According to the WHO classification, our cases were divided into three subtypes: the well differentiated *indolent plasmacytoma*, in one cat and 19 dogs; the locally aggressive *anaplastic*

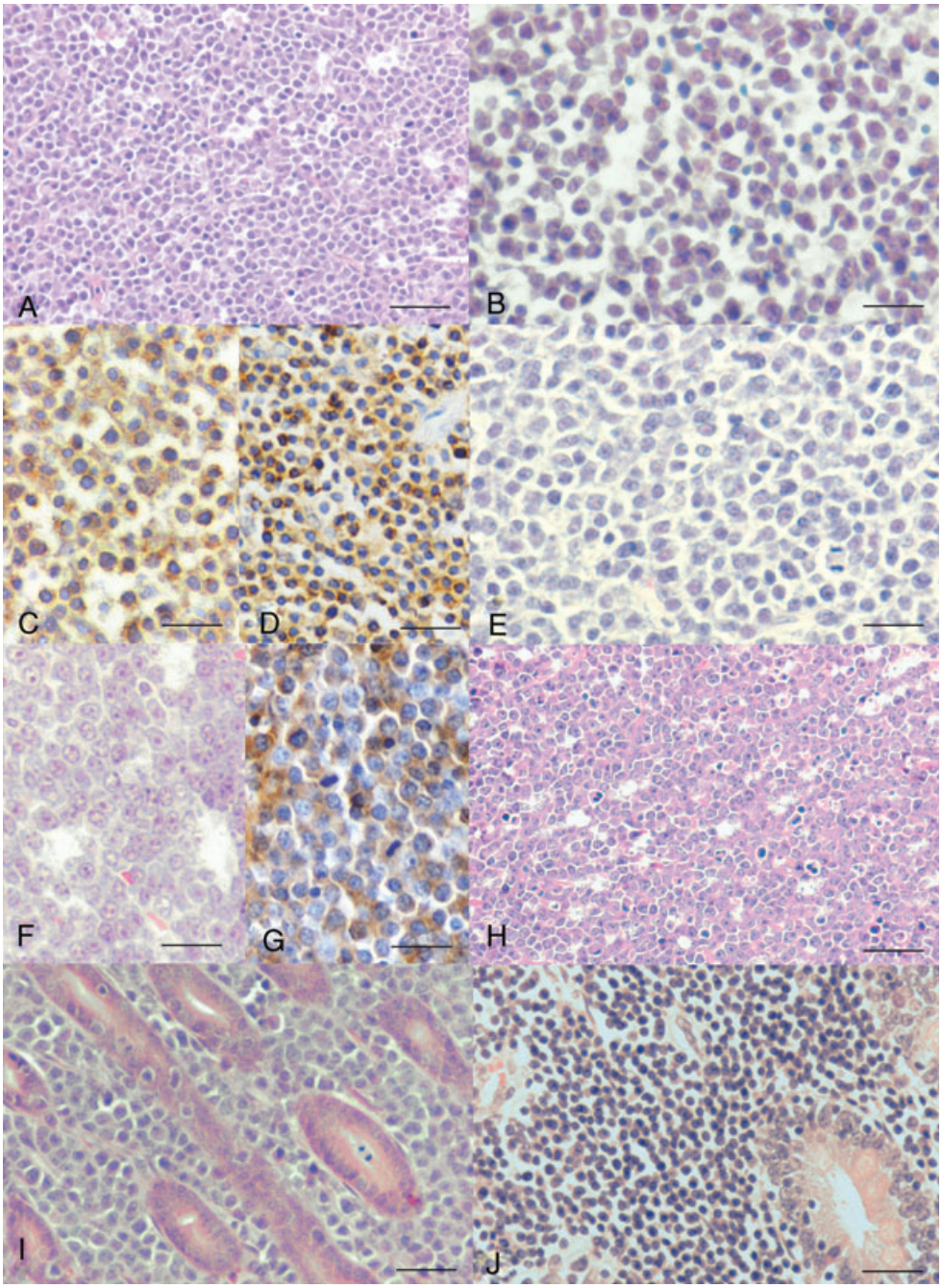

Figure 1. Lymph node: dog. (A) B-LBL with starry-sky appearance, resembling Burkitt's lymphoma. HE. Bar = 25 μm. (B) Plasma cell tumour (indolent). HE. Bar = 50 μm. (C) Plasma cell tumour (indolent). Immunohistochemistry CD79a counterstained with Papanicolau haematoxylin. Bar = 50 μm. (D) Plasma cell tumour (indolent). Immunohistochemistry CD3 counterstained with Papanicolau haematoxylin. Bar = 50 μm. (E) B-LCL classified as DLBCL with mixed pattern given by immunoblasts and centroblasts. HE. Bar = 50 μm. (F) LCIBL characterized by immunoblasts and plasmacytoid morphology. HE. Bar = 50 μm. (G) LCIBL with plasmacytoid morphology. Immunohistochemistry CD79a counterstained with Papanicolau hematoxylin. Bar = 50 μm. (H) Burkitt's lymphoma with starry-sky appearance resulting from the presence of numerous tingible bodies laden macrophages. HE. Bar = 25 μm. Jejunum: dog. (I) ITCL characterized by intraepithelial large neoplastic cells invading the adjacent mucosa. HE. Bar = 50 μm. (J) ITCL characterized by small neoplastic cells. HE. Bar = 50 μm.

plasmacytoma, in one cat and one dog; and *plasma cell myeloma*, in one cat. Two plasmacytomas were positive for both CD79 and CD3 (Fig. 1B–D).

B-large cell neoplasms

Large B-cell lymphoma (*B-LCL*) was diagnosed in 56 dogs and 8 cats. The commonest subtype was the *diffuse large B-cell lymphoma*, *cleaved* or *non-cleaved* (*DLBCL* or *DLBCCL*). It was observed in 41 dogs and 5 cats and was characterized by scanty cytoplasm, vesicular nuclei, branched chromatin and two or three prominent nucleoli (Fig. 1E). *Large cell immunoblastic lymphoma* (*LCIBL*) was observed in 13 dogs and 3 cats and displayed round, oval or cleaved nuclei, with reticular chromatin, branched or peripheral onto the nuclear membrane, and a large central nucleolus. The starry-sky pattern was a frequent finding in this subtype (Fig. 1F,G).

As for less common B-LCL subtypes, one dog presented *T-cell rich B-cell lymphoma*, characterized by large malignant B-cells surrounded by a majority of reactive small and cleaved T-lymphocytes, associated with a fine and diffuse reticular sclerosis pattern. Furthermore, one case *thymic (mediastinal) B-cell lymphoma* was found in the dog, depicting large cells with vesicular and cleft nuclei and prominent nucleoli.

Other lymphoid neoplasms

One canine case *of Burkitt-like lymphoma* was characterized by a monomorphic proliferation of very uniform, round or oval cells with vesicular nuclei of constant size, thickening of the nuclear membrane, multiple and small nucleoli, a narrow ring of cytoplasm and a mosaic pattern given by closely interfaced, distinct cellular boundaries and moderate anisokaryosis (Fig. 1H).

Two canine cases of *cutaneous epitheliotropic lymphoma* (*CEL*) were characterized by small (and cleaved in one case) lymphocytes of intermediate size, with abundant, irregular, 'fish-head' silhouette and clear cytoplasm. These cells colonized the epithelial layers (Pautrier microabscesses). Tumour tissues in both cases were vascularized by capillaries with epithelioid endothelium. In *cutaneous non-epitheliotropic lymphoma (CNEL)*, diagnosed in four dogs, neoplastic cells displayed clear cytoplasm and small, irregularly indented, hyperchromatic nuclei, extending to dermis and largely to panniculus.

Table 4. Tumour topography in 171 cases of lymphoma.

Anatomic form	Dog	Cat	Total
Multicentric	90	9	99
Alimentary	9	25	34
Cutaneous	16	8	24
Splenic	6	–	6
Hepatic	1	–	1
Renal	–	2	2
Mediastinal	–	1	1
Unknown	1	3	4
	123	48	171

Extranodal or peripheral T-cell lymphoma (*PTCL*) was observed in 11 cats and 3 dogs. These cases presented subcutaneous, heterogeneous, malignant proliferation of neoplastic cells, which varied from small to large, with variable amount of cytoplasm and irregularly cleaved nuclei. *Angiotropic lymphoma* (*ATL*) was observed in three cats, because of the presence of dense lymphoid cuffs around small vessels (primarily small arteries).

Intestinal T-cell lymphoma (*ITCL*) was detected in 16 cats and 5 dogs. Our ITCL series was characterized by darkly stained and irregular cells with marked anisocytosis, invading the intestinal epithelium and associated with neoangiogenesis (Fig. 1I,J). From an anatomical perspective, muticentric lymphoma prevailed in dogs (90/123; 73.2%) and gastrointestinal lymphoma in cats (25/48; 52.1%). All the correlations between species and tumour topography are summarized in Table 4.

Feline alimentary lymphomas included, (T-cell types), 16 ITCL, 1 ATL and 4 PTCL. Feline alimentary lymphoma of the B-cell lineage were classified as one LBL (B-LBL), four LCL (B-LCL) and one MALT lymphoma. The mean age of cats with alimentary T-cell lymphoma was 8.3 years, while for those with alimentary B-cell lymphoma it was 9.3 years.

Discussion

Our findings partially reflect the published literature. Multicentric lymphoma represented 73.2% of canine lymphomas (reference data: 84%)[14] and alimentary lymphoma represented 52.1% of feline

lymphomas (reference data: 26%)[8] in this study. In our series, the commonest category in feline alimentary lymphoma was ITCL (64%; 16 out of 25), in contrast to other studies that report mainly B-cell lymphomas.[8,15]

In dogs we recorded a strong prevalence of B-cell lymphomas (97/123; 79.9%), as observed by others.[16] In contrast, in cats we found a prevalence of T-cell lymphomas (31/48; 64.6%), which contradicts reports in the literature. A possible explanation could be that previous studies underestimated the number of T-cell lymphomas because of the lack of immunostaining.[8] In canine, as in human medicine, the vast majority of lymphoid neoplasms (80–85%) is of B-cell origin.[17] The inherent genomic instability of B-cell germinal centres might explain why they are more susceptible to lymphomas than mature T-cells, which have fixed and stable T-cell receptor genes. The T-cell lymphoma prevalence in our feline samples does not seem related to old age as suggested by some reports.[10] Furthermore, the mean age of cats with B- or T-alimentary lymphoma did not differ significantly (9.3 versus 8.3 years).

As observed by others,[18] B-LCLs was the commonest type of B-cell lymphoma in both dogs (B-LCL: 56/97; 57.7%) and cats (8/17; 47.1%).

T-cell lymphomas were predominantly of the T-lymphoblastic (T-LBL) type in dogs (8/26; 30.8%), while ITCL (16/31; 51.6%) and PTCL (11/31; 35.5%) prevailed in cats. These findings are in agreement with published data, although the ITCL was less represented.[8,15]

Some classes of the WHO classification of lymphomas are defined by their clinical behaviour or growth pattern rather than by morphology. This is the case of regular small non-cleaved B-cells with a moderate amount of highly basophilic cytoplasm, that could be *B-LBL*, *Burkitt-type lymphoma* or *high-grade B-cell lymphoma Burkitt-like*, according to the WHO typing. The two classes of Burkitt lymphoma are characterized by the frequent presence of a starry-sky appearance resulting from the presence of numerous tingible body macrophages (Fig. 1A,H), while this is only an occasional feature in *B- or T-LBL*. *Burkitt-type* has polygonal cell shape and more regular nuclei, when compared with *Burkitt-like lymphomas* (formerly known as small non-cleaved B-cell lymphoma).[9,10] These two subtypes are discussed here, as some authors consider them as representing a unique class.[19] B-LBL can be distinguished from Burkitt lymphoma by five criteria: (1) smaller nuclei, (2) thicker nuclear membrane, (3) absence of parachromatin clearing (dispersed or powder-like pattern) (4) prominent multiple (2–5) small but well-defined nucleoli, located beneath the nuclear membrane and (5) negative reaction to acid phosphatase on imprints. The subset of lymphomas that stained negatively to both CD79 and CD3 were composed of small non-cleaved cell lymphomas with high mitotic rate and numerous apoptotic cells. This lack of immunoreactivity may be the result of a weak antigen expression. This happens to precursor cell lymphomas, where early pre-T-cell tumours are usually negative for CD3, CD4 and CD8, as described in human medicine.[17]

Centrocyte and centroblast are centrofollicular cells, and may spread out in both follicular and diffuse tumours. Nevertheless, in diffuse lymphoma they should more properly be defined as 'centrocyte-like' or small cleaved cells and 'centroblast-like' or large cleaved and not cleaved cells.[5] In the WHO classification, the terms centrocytes and centroblasts have been superceded by the term monocytoid B-cells, as centrocytes and centroblasts can be also present in follicular MZLs, such as MALT-L, when the marginal neoplastic cells surround and shrink the follicular centre cells.

DLBCL includes LCIBL and DLBCL or DLBCCL. The morphology of these subtypes is at times intermediary and a mixed population of immunoblasts and centroblasts is commonly observed. Centroblastic lymphoma can evolve to the immunoblastic type (Fig. 1E).[5] For these reasons WHO classification allows the inclusion of proliferation of centroblasts and immunoblasts in the same category, although the possibility of discrimination still remains.[9,10]

Animal lymphomas are more frequently diffuse than in humans,[11] as confirmed in our series. This difference between animal and human lymphoma had been attributed to the late veterinary diagnosis, which allows that follicular tumours may diffuse.

Lymphocyte size was not always coherent to what was expected. Notably, we observed large cells

resembling centrocytes, and small to medium cells resembling immunoblasts. This latter type has been described as typical in canines, but not mentioned in the WHO classification.[9,20]

B-cell thymic (mediastinal) lymphoma is described in the WHO classification, while T-cell thymic lymphoma is not taken into consideration, despite its higher frequency. Between the precursor lymphomas, only T-cell leukaemias of thymic origin are quoted. T-cell thymic lymphoma is a lymphoblastic proliferation that rapidly peripheralizes to bone marrow and moves to become acute leukaemia.[9]

We observed one *B-cell mediastinal lymphoma* in a young dog. This is a rare neoplasm of large breed dogs that cannot be properly defined without the topographical association defined in the WHO system of classification.

Plasma cells tumours were considered by WHO as lymphomas. We observed 12 cases of lymphomas in dogs and one case in a cat, characterized by predominance of immunoblasts or centroblasts with plasmacytoid differentiation (Fig. 1F,G). We considered these cases respectively as LCIBL or DLBCL, but these plasmablastic lymphomas should be classified independently. Apart from multiple myeloma, which has a distinctive bone marrow involvement, WHO classification discriminates only two subtypes of extramedullary plasmacytoma: indolent and anaplastic, although the existence of different histological patterns, corresponding to many prognostic classes, was demonstrated.[21] In human medicine, there is confusion on the same topic: *LPL* is elsewhere called *Immunocytoma*, in both cases constituted by small cells associated with immunoglobulin production as in Waldenström syndrome.[22]

Plasmacytoid tumours can co-express both B- and T-phenotypes, at least on immunohistochemical grounds. In our collection, two cases of plasmacytoma were positive for CD79 and CD3. In a recent study reporting several cases of T-cell lymphomas with plasmacytoid morphology, the recognition of a new provisional WHO entity has been discussed.[10,23]

According to our experience, extramedullary plasma cell tumours with systemic involvement are almost as frequent, since 9 out of 18 plasmacytomas in dogs and 1 out of 3 in cats were pure nodal, without any apparent mucocutaneous or hepatosplenic involvement.

In our experience, anaplastic plasmacytoid tumours are more easily diagnosed on cytological samples, than on histological sections, since the crowded cells tend to lose their typical morphology.

Some cases of follicular B-cell tumours and in other T-cell lymphomas, recognized for their morphology, were immunohistochemically negative. This could be because of a receptor loss during the retro-differentiation or the anaplastic evolution of the original clone. Conversely, some studies show a co-expression of CD79 and CD3, suggesting a molecular biology approach to obtain phenotypic data.[18,24–26]

Two multicentric and nodal T-lymphocytic lymphomas were seen in our study. These cases could not be classified according to the tumour topography as suggested by the WHO classification, since these cases were CD3 positive and CD79 negative, they were not leukaemic and they had no cytoplasmic granules. SLL of T-cell type are not included in the WHO classification,[9] as such neoplasms are considered by other relevant publications.[10] We classified them as PTCL, in order to follow strictly the WHO classification. In human oncology PTCL was divided into *nodal* and *extranodal*, our two cases could be considered nodal PTCL.[10,22,27] Again, between PTCL there are lymphomas arising from vaccination sites.[9] According to some authors, PTCL and B-LCL play the role of a 'catch all' category for the unclear cases respectively of T and B-cell lymphoma.[28] The WHO classification suggests to define them as not otherwise specified (NOS) lymphomas.

When localized, *CEL*, observed in two cases, is the counterpart of the human *mycosis fungoides*, and, if leukaemic [mycosis fungoides/Sezary syndrome (*MF/SS*)], it is the counterpart of the *Sezary syndrome*. In human medicine other clinical presentations are reported: *pagetoid reticulosis* or *Woringer-Kolopp* disease. CEL and ITCL both show two specific features: epithelial invasion and neoangiogenesis.

ITCL was observed in 16 out of 48 feline lymphomas. This high prevalence of alimentary lymphoma in cats, in comparison with other topographic presentations, could be related to the

high number of intestinal surgeries more frequently accepted by the pet owners in our country, in comparison with other surgeries. This category describes a proliferation of small[9] or medium to large[29] darkly stained T-cells, with irregular nuclear contours invading the intestinal mucosa. The main cell types can be small lymphocyte as well as immunoblasts and the diagnostic criteria to follow are represented by the gastrointestinal topography and the mucosa invasion (Fig. 1I,J).[10,30,31] The *inflammatory* (rich in inflammatory cells) or *lymphoid* (more heterogeneous) ITCL were not evident in our series.

For a correct diagnosis, the LGL lymphoma needs cytology or further immunohistochemical characterization, such as CD3, CD4, CD8, supported by CD11b, CD18, CD103, perforin and specific esterase activity. The single case here reported was unrecognizable on histological section and needed a cytological examination from smeared cells, rich in the typical granules.[32,33]

The ATL class is currently divided into two subclasses, angioimmunoblastic lymphoma (AIL) and angiocentric lymphoma (ACL). In our opinion, the peculiar cell disposition of the latter ACL does not derive from a real vascular tropism, but represent fragments of tissue survived where a sufficient oxygenation is granted, as a remnant of the cell death that takes place far from the vessels. The pathogenesis of that subtype should be better characterized.

In our opinion, these observations could be useful for an evolution of the WHO classification. In human medicine, some critical points of the former WHO lymphoma classification were solved.[22,27]

In the WHO classification, one of the main diagnostic pitfalls for pathologists remains the subtle distinction between nodal inflammation and neoplasms. In benign follicular lymphoid hyperplasia, germinal centres with polarity may extend from the outer cortex to the medulla. The cell types within the germinal centres are heterogeneous. At low magnification, activated lymphocytes may cross over the capsule, involving the extranodal tissues, although the subcapsular sinuses remain free. The number of *antigen presenting cells* (APC) may indicate an inflammatory reaction. In the follicle, dendritic or interdigital cells have very clear and abundant water-clear cytoplasm and must be distinguished from large neoplastic cells. Furthermore, these APCs are S-100 positive. Lymphomas of the alimentary tract are often associated with inflammatory reaction. T-cell lymphoma, moreover, often presents inflammatory foci.[9] At last, retroviral infections tends to show both lymphomatous proliferations and inflammatory reactions.[10,34]

Finally, we did not diagnose *hairy cell leukaemia*; generally this subtype of neoplasm is not recognized in animals.[9]

Conclusions

The WHO human classification has been adapted to veterinary medicine. However, further co-operative studies between clinicians and pathologists should be performed, in order to improve the effectiveness of the classifications. In some cases, no category fits animal lymphomas and some categories are not represented in veterinary medicine. In addition, a further separation into specific classifications for canine and feline is required.

References

1. Berhard CW and Hutchison RE. The problem of classifying lymphomas: an orderly prescription for progress. *Annals of Oncology* 1997; **8**(Suppl. 2): S3–S9.
2. Parodi AL. Classification of malignant lymphoma in domestic animals: history and conceptual evaluation. *European Journal of Veterinary Pathology* 2001; **7**: 43–50.
3. Cogliatti SB and Schmid U. Who is WHO and what is REAL? A review of the new WHO classification (2001) for malignant lymphomas. *Swiss Medical Weekly* 2002; **132**: 607–617.
4. Diebold J. La nouvelle classification des tumeurs des tissus hematopoietiques et lymphoides de l'oms: hemopaties lymphoides, histiocytaires et mastocytaires. *Hematologie* 2002; **8**: 46–51.
5. Lennert K. *Malignant Lymphomas Other than Hodgkin's Disease*, Berlin, Heidelberg, New York, Springer-Verlag, 1978.
6. The non-Hodgkin's lymphoma pathologic classification project. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. *Cancer* 1982; **49**: 2212–2135.

7. Stansfeld AG, Diebold J, Kapanci Y, Kenenyi G, Lennert K, Mioduszeuskz O, Noel H, Rilke F, Sundstrom C, Van Unnik JAM and Wright DH. Updated Kiel classification for lymphomas. *Lancet* 1988; **331**: 292–293.
8. Valli VE, Jacobs RM, Norris A, Couto CG, Morrison WB, McCaw D, Cotter S, Oglivie G and Moore A. The histologic classification of 602 cases of feline lymphoproliferative disease using the National Cancer Institute Working Formulation. *Journal of Veterinary Diagnostic Investigation* 2000; **12**: 295–306.
9. Valli VE, Jacobs RM, Parodi AL, Vernau W and Moore PF. *Histological Classification of Hematopoietic Tumors of Domestic Animals*, Washington D.C., Armed Forces Institute of Pathology, 2002.
10. Valli VE. *Veterinary Comparative Hematopathology*, Ames, Blackwell Publishing, 2007.
11. Vonderhaar MA and Morrison WB. Lymphosarcoma. In: *Cancer in Dogs and Cats: Medical and Surgical Management*, WB Morrison, ed., Jackson, Teton New Media, 2002.
12. Fournel-Fleury C, Ponce F, Felman P, Blavier A, Bonnefont C, Chabanne L, Marchal T, Cadore JL, Goy-Thollot I, Ledieu D, Ghernati I and Magnol JP. Canine T-cell lymphomas: a morphological, immunological and clinical study of 46 new cases. *Veterinary Pathology* 2002; **39**: 92–109.
13. Pittaluga S, Bijnens L, Teodorovic I, Hagenbeek A, Meerwaldt JH, Somers R, Thomas J, Noordijk EM and DeWolf-Peeters C. Clinical analysis of 670 cases in two trials of the European Organization for the Research and Treatment of Cancer Lymphoma Cooperative Group subtyped according to the revised European-American classification of Lymphoid neoplasms: a comparison with the Working Formulation. *Blood* 1996; **87**: 4358–4367.
14. Jacobs RM, Messick JB and Valli VE. Tumors of the hemolymphatic system. In: *Tumors in Domestic Animals*, DJ Meuten, ed., Ames, Iowa Sate Press, 2002.
15. Patterson-Kane JC, Perrins Kugler B and Francis K. The possible prognostic significance of immunophenotype in feline alimentary lymphoma: a pilot study. *Journal of Comparative Pathology* 2004; **130**: 220–222.
16. Ponce F, Marchal T, Magnol JP and Fournel-Fleury C. Canine malignant lymphomas: a morphological, immunological and epidemiologic study of 608 new cases. Comparison with human's non-Hodgkin's lymphomas. *Proceedings of 22nd Meeting E.S.V.P. (European Society of Veterinary Pathology)*, Olsztyn, Poland, 15–18 September, 2004, 33 B.
17. Aster JC. Diseases of white blood cells, lymph nodes, spleen and thymus. In: *Robbins and Cotran Pathologic Basis of Disease*, 7th edn., V Kumar, AK Abbas and N Fausto, eds., Philadelphia, Elsevier Saunders, 2004: 661–709.
18. Burnett RC, Vernau W, Modiano JF, Olver CS, Moore PF and Avery AC. Diagnosis of canine lymphoid neoplasia using clonal rearrangements of antigen receptor genes. *Veterinary Pathology* 2003; **40**: 32–41.
19. Pileri SA, Ascani S, Sabattini E, Fraternali Orcioni G, Poggi S, Piccioli M, Piccaluga PP, Gamberi B, Zinzani PL, Leoncini L, Falini B. The pathologist view point. Part I–indolent lymphomas, part II–aggressive lymphomas. *Hematologica* 2000; **85**: 1291–1307, 1308–1321.
20. Fournel-Fleury C, Magnol JP, Chabanne L, Ghernati I, Marchal T, Bonnefond C, Byron PA and Felman P. Growth fractions in canine non-Hodgkin's lymphomas as determined in situ by the expression of the Ki-67 antigen. *Journal of Comparative Pathology* 1997; **117**: 61–72.
21. Platz SJ, Breuer W, Pfleghaar S, Minkus G and Hermanns W. Prognostic value of histopathological grading in canine extramedullary plasmacytomas. *Veterinary Pathology* 1999; **36**: 23–27.
22. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA and Bloomfield CD. The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Hematology Journal* 2000; **1**: 53–66.
23. Ponce F, Magnol JP, Marchal T, Chabanne L, Ledieu D, Bonnefont C, Felman P and Fournel-Fleury C. High-grade canine T-cell lymphoma/leukemia with plasmacytoid morphology: a clinical pathological study of nine cases. *Journal of Veterinary Diagnostic Investigation* 2003; **15**: 330–337.
24. Moore PF, Woo JC, Vernau W, Kosten S and Graham PS. Characterization of feline T cell receptor gamma (TCRG) variable region genes for molecular diagnosis of feline intestinal T cell lymphoma. *Veterinary Immunology and Immunopathology* 2005; **106**: 167–178.
25. Pilozzi E, Pulford K, Jones M, Müller-Hermelink HK, Falini B, Ralfkiaer E, Pileri S, Pezzella F, De Wolf-Peters C, Arber D, Stein H, Mason D and Gatter K. Co-expression of CD79a (JCB117) and CD3 by lymphoblastic lymphoma. *Journal of Pathology* 1988; **186**: 140–143.
26. Wilkerson MJ, Dolce K, Koopman T, Shuman W, Chun R, Garrett L, Barber L and Avery A. Lineage

differentiation of canine lymphoma/leukemias and aberrant expression of CD molecules. *Veterinary Immunology and Immunopathology* 2005; **106**: 179–196.
27. Pileri SA, Fraternali Orcioni G, Ascani S, Milani M, Leoncini L and Falini B. Classificazione dei linfomi maligni: l'istopatologia quale guida alla terapia. *Atti del 37° Congresso Nazionale della Società Italiana di Ematologia*, Torino, 26–27 Settembre, 1999.
28. Feller AC and Diebold J. *Histopathology of Nodal and Extra-Nodal Non-Hodgkin's Lymphomas*, Berlin, Heidelberg, Springer-Verlag, 2004.
29. French RA, Seitz SE and Valli VE. Primary epitheliotropic alimentary T-cell lymphoma with hepatic involvement in a dog. *Veterinary Pathology* 1996; **33**: 349–352.
30. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B and Gatter KC. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; **84**: 1361–1392.
31. Harris NL. A practical approach to the pathology of lymphoid neoplasm: a revised European-American classification from the International Lymphoma Study Group 9. In: *Important Advances in Oncology*, VT DeVita, S Hellman and SA Rosenberg, eds., Philadelphia, Lippincott, 1995: 111–140.
32. Roccabianca P, Vernau W, Caniatti M and Moore PF. Feline large granular lymphocyte (LGL) lymphoma with secondary leukemia: primary intestinal origin with predominance of a CD3/CD8$\alpha\alpha$ phenotype. *Veterinary Pathology* 2006; **43**: 15–28.
33. Young NA and Al Saleem T. Diagnosis of lymphoma by fine-needle aspiration cytology using the revised European-American classification of lymphoid neoplasms. *Cancer* 1999; **87**: 325–345.
34. Callanan JJ, Jones BA, Irvine J, Willett BJ, McCandlish IAP and Jarret O. Histologic classification and immunophenotype of lymphosarcomas in cats with naturally and experimentally acquired feline immunodeficiency virus infection. *Veterinary Pathology* 1996; **33**: 264–272.
35. Madewell BR, Gieger TL, Pesavento PA and Kent MS. Vaccine site-associated sarcoma and malignant lymphoma in cats: a report of six cases (1997–2002). *Journal of the American Animal hospital Association* 2004; **40**: 47–50.
36. Lucke VM. Primary cutaneous plasmacytomas in the dog and cat. *Journal of Small Animal Practice* 1987; **28**: 49–55.
37. Ponce F, Magnol JP, Ledieu D, Marchal T, Turinelli V, Chalvet-Monfray K and Fournel-Fleury C. Prognostic significance of morphological subtypes in canine malignant lymphomas during chemotherapy. *Veterinary Journal* 2004; **167**: 158–166