Polish Journal of Veterinary Sciences Vol. 18, No. 1 (2015), 163–169

DOI 10.1515/pjvs-2015-0021

Original article

Large granular lymphoma in six cats

R. Sapierzyński[1], U. Jankowska[2], D. Jagielski[2], K. Kliczkowska-Klarowicz[1]

[1] Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Nowoursynowska 159c, 02-766 Warsaw, Poland
[2] Białobrzeska Veterinary Surgery in Warsaw, Poland

Abstract

Large granular lymphomas (LGLs) comprise a specific group of lymphomas regardless of classification scheme. An LGL consists of cells that show less or more mature morphology, but typically neoplastic cells possess cytoplasmic azurophilic granules clearly visible during cytological examination. The aim of the present study was to present clinical and cytological data on large granular lymphomas in cats and to analyses the therapeutic responses in treated cases. During the period from 2012 to 2014 six cats were as having large granular lymphoma. In one cat a nasal form of LGL was recognized, a systemic form was recognized in another cat, and in four cases an alimentary form was recognized. Cellular samples for cytopathology were collected from the cat with nasal cavity mass, from the enlarged mandibular lymph node and thoracic cavity from second cat, and in four cats from the abdominal mass during ultrasound-assisted fine-needle biopsy. Therapy was introduced in 5 of the 6 cats. In two cases palliative therapy with glucocorticoids was conducted, in two cases chemotherapy with COP protocol, and therapy with masitinib in one case. The median of survival time for cats treated with anticancer therapy was 9 months, the median of survival time for cats treated with glucocorticoids was 1.5 months. In conclusion, large granular lymphomas, especially the alimentary form, are a relatively common type of lymphoma in cats. Simple diagnostic methods such as clinical examination, imaging techniques and routine cytology are sufficient in majority of cases. Despite aggressive behavior and poor general prognosis, conventional chemotherapy lead to a good response in some treated cats regardless of anatomic form and histologic grade of malignancy.

Key words: cat, cytology, large granular lymphocyte, large granular lymphoma

Introduction

Lymphomas are the most common malignant tumors recognized in cats, accounting for about 50-90% of hematopoietic tumors and for approximately 20-25% of all neoplasms in this animal species. Large granular lymphomas (LGLs) comprise a specific group of lymphomas regardless of classification scheme. An LGL consists of cells that show less or more mature morphology, but typically neoplastic cells possess cytoplasmic azurophilic granules clearly visible during cytological examination. It seems that LGLs are tumors that are not commonly recognized in cats because they comprise only 6-10% of alimentary lymphomas (Krick et al. 2008, Pohlman et al. 2009, Moore et al. 2012). On the other hand, nowadays alimentary lymphoma is the most common anatomic form of lymphomas in cats. Additionally, in one

Correspondence to: R. Sapierzyński, e-mail: rafal_sapierzynski@sggw.pl, tel.: +48 22 593 61 53

study LGLs comprised more than 14% of all lymphomas recognized in cats, making LGL a quite common problem in feline oncology (Chino et al. 2013). Among other felids lymphoma composed of large granular lymphocytes involving ocular structures was also described in a caracal (*Caracal caracal*; Aitken-Palmer et al. 2011).

Based on analysis of described cases there is no relationship between LGL development and viral infection, although some cats with these lymphomas were infected with feline leukemia virus or feline immunodeficiency virus (Wellman et al. 1992, Endo et al. 1998, Roccabianca et al. 2006, Krick et al. 2008). It was suspected that, similarly as was confirmed in human patients, feline LGLs can be a consequence of long-standing inflammatory bowel disease (IBD). In one study including 20 cats with LGLs, medical history revealed the possibility of previous chronic IBD in 7 of these patients (Roccabianca et al. 2006). It is possible that LGL develops as a consequence of neoplastic transformation of a clone of intraepithelial lymphocytes chronically stimulated during the inflammatory process.

LGLs originate from T cytotoxic (CD3 positive) or natural killer (CD3 negative) lymphocytes, cells which normally have cytoplasmic granules, containing various biologically active substances including granzyme, perforins, acid phosphatase and others (Kariya et al. 1997, Neta et al. 2008, Tsuboi et al. 2010). In the majority of cases LGLs develop in intestinal mucosa producing segmental enlargement of the intestine, which with time progress into a tumoral mass easily detected during clinical examination. Mesenteric lymph nodes are also typically affected, but abdominal internal organs are not commonly infiltrated by neoplastic cells.

Feline LGLs are not commonly described in veterinary literature, with only few articles including large groups of cats being available. The aim of the present study was to present clinical and cytological data on large granular lymphomas in six cats and to analyse the therapeutic responses in treated cases.

Materials and Methods

This retrospective study was conducted on cats which were patients of the Białobrzeska Veterinary Surgery in Warsaw; the cases were identified retrospectively by searching the medical records for the period 2012-2014. Data on signalement, clinical signs, results of hematologic examination, biochemistry and imaging techniques were recorded in each case. Microscopic examination was carried out in the Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Live Sciences (SGGW). Cellular samples of solid masses were collected by fine-needle aspiration biopsy of directly visible pathological masses or ultrasonographic-assisted fine-needle aspiration biopsy from a lesion/lesions detected during imaging techniques (thoracic or abdominal radiography or abdominal ultrasonography). In cases with presence of serosal effusions, fluid from the serosal cavity was collected by thoracocentesis or abdominocenthesis, placed into an EDTA tube, and then centrifuged. Sediment was used as material for smears. Cellular samples of any kind were subsequently smeared in a routine manner, dried and processed. For cytopathological examination at least 3 cytologic smears of good quality were dried, fixed in 70% methanol, stained with Giemsa solution and examined by light microscope. Diagnosis of large granular lymphoma was made based on widely accepted cytologic criteria, assuming that cells had the morphology of mature or immature lymphocytes, with round or slightly indented single nuclei, moderate to voluminous cytoplasm and with fine to large azurophilic granules (Roccabianca et al. 2006). Additionally, data on treatment mode, time of therapy, time from diagnosis to death and cause of death were also recorded.

Results

During the period from 2012 to 2014 six cats were recognized as having large granular lymphoma. In one cat a nasal form of LGL was recognized (case no. 3); clinically sneezing and nasal serous discharge stained with blood was present at the beginning of the disease. Finally, face deformity and a pink, fleshy mass protruding from the right nasal cavity was present in the advanced stage. In other cat (case no. 4) a systemic form was recognized with nonspecific clinical signs: dyspnea, thoracic effusion and mandibular, mesenteric, and mediastinal lymphadenomegaly. In four cases an alimentary form was recognized (cases 1, 2, 5, 6). Mild to severe intensity of clinical signs related to the alimentary tract (lack of appetite, vomiting, diarrhea, loss of body mass) were noted; additionally, an abdominal mass was detected during abdominal cavity palpation in each of these animals. Particular data on signalement, imaging techniques used, main pathology detected, cell blood count and biochemistry are presented in Table 1. Cellular samples for cytopathology were collected from the nasal cavity mass (cat with nasal form), from the enlarged mandibular lymph node and the thoracic cavity (cat with systemic form) and from the abdominal mass during ultrasound-assisted fine-needle biopsy (four

Table 1. Data on signalement, imaging techniques used, main pathology detected, cell blood count and biochemistry of six cats with large granular lymphoma.

No	Sex	Age year	Imaging techniques	Main pathology	leu	ery	cre	Liver enzymes	Protein
1	♂	16	Abdominal ultrasonography	Mesenteric lymphadenomegaly	N	L	H	H	N
2	♂	9,5	Abdominal ultrasonography	Mesenteric lymphadenomegaly Intestinnal mass	N	N	N	H	N
3	♂	13	Head computed tomography	Nasal cavity mass	H	N	N	N	N
4	♀	11	Chest ultrasonography	Mandibular lymphadenomegaly Pleural effusion	H	N	N	N	N
5	♀	12	Abdominal ultrasonography	Mesenteric lymphadenomegaly Thickening of small intestine	N	N	N	H	N
6	♀	11	Abdominal ultrasonography	Mesenteric lymphadenomegaly hickening of small intestine	L	N	N	N	N

Legend: N – normal; H – increased; L – decreased; leu – leukocyte number; ery – erythrocyte number; cre – creatinine concentration).

Table 2. Results of cythopatological examination of six cats with large granular lymphoma.

No	General cellularity	Cytoplasmic granules		Cellular morphology	Mitoses	Numerous other leukocytes
		size	distribution			
1	High	Fine	Diffuse	Immature	No	Yes
2	Moderate	Large	Diffuse	Predominantly mature	No	Yes
3	High	Fine	Grouped	Immature	Yes	Yes
4	Moderate	Large	Diffuse	Mature	No	No
5	High	Fine	Grouped	Mature	Yes	Yes
6	High	Fine	Grouped	Predominantly mature	Yes	Yes

Table 3. Particular data on mode of therapy, response, time from diagnosis to death, and cause of death in six cats with large granular lymphoma.

No	Therapy	Duration of therapy	Effect of therapy	Time from diagnosis to death	Cause of death
1	GKS	1.5 months	No response	1.5 months	Euthanasia – progression
2	GKS	2 months	No response	At least 2 months	No information
3	COP	8 months	Firstly, reduction of size of mass	9 months	Euthanasia – progression
4	Masitinib	3 months	Stabilization of disease	6 months	Hepatic insufficiency
5	COP	12 months	Firstly, reduction then stabilization of mass size	16 months	Euthanasia – progression
6	No therapy	–	–	At least 1.5 months	No information

Legend: GKS – glucocorticoids; COP – cyclophosphamide, vincristine, prednisone.

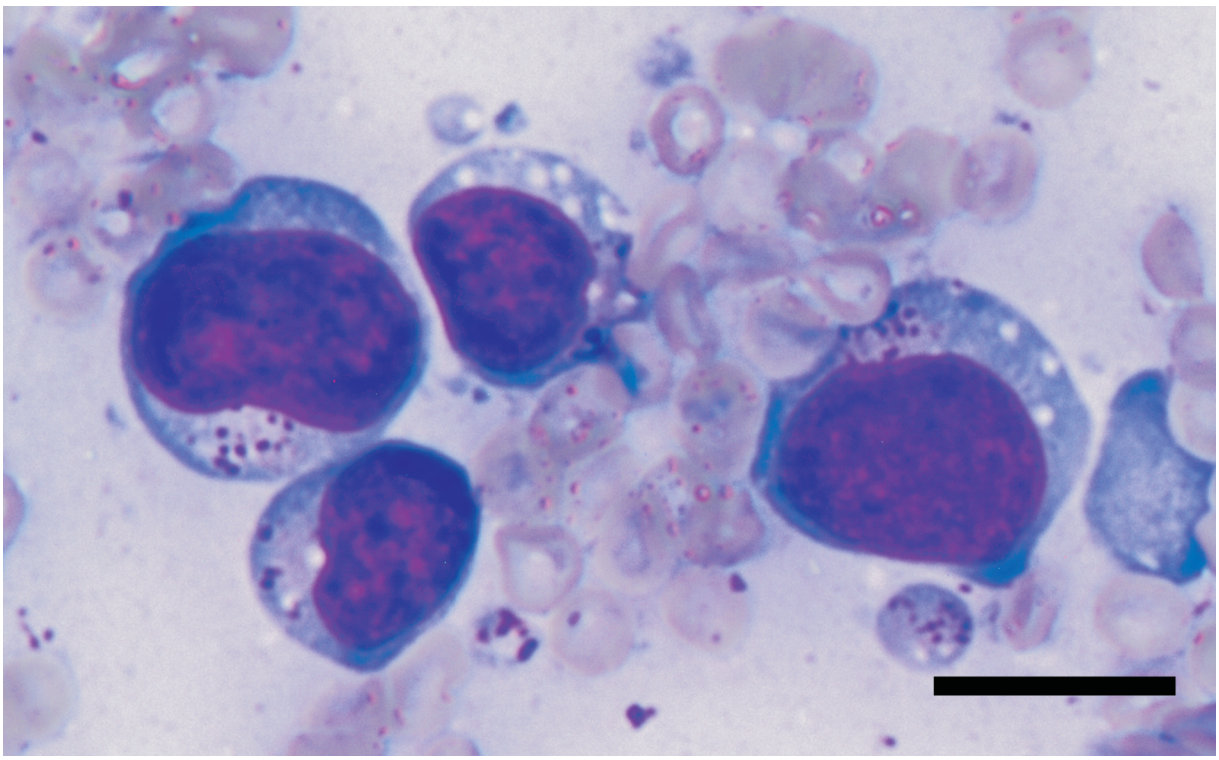

Fig. 1. Large granular lymphoma, fine-needle biopsy of nasal mass, cat (No. 3). Neoplastic lymphocytes have immature morphology, characterized by large size, abundant basophilic cytoplasm, and some cells possess fine cytoplasmic vacuoles. Granules are small and grouped in one area of cytoplasm. Giemsa stain, bar = 20 μm.

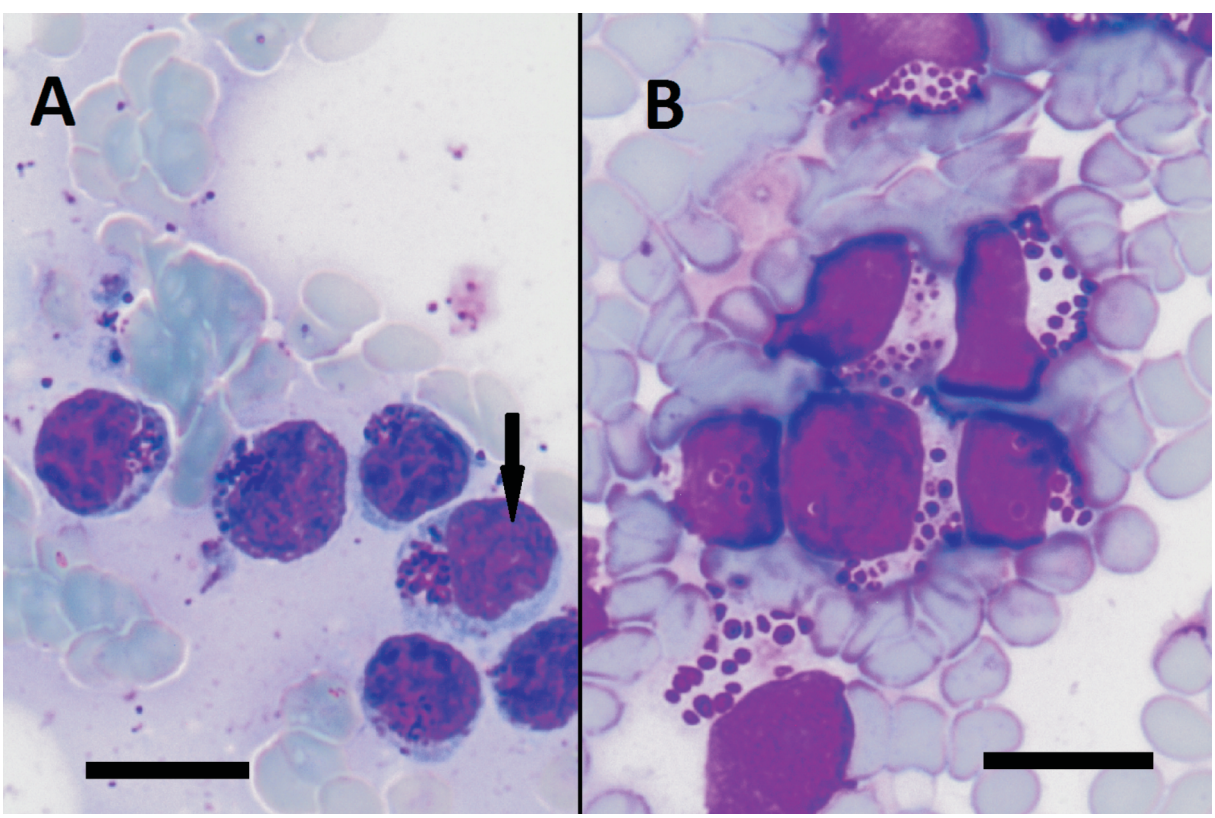

Fig. 2. Large granular lymphoma. (**A**) Fine-needle biopsy of abdominal mass, cat (No. 6). Neoplastic lymphocytes have predominantly mature morphology (scant, light cytoplasm, and condensed chromatin), one cell is less mature (arrow indicates nucleolus); prominent cytoplasmic granules are fine and grouped. Giemsa stain, bar = 20 μm. (**B**) Fine-needle aspiration biopsy of abdominal mass, cat (No. 2). Neoplastic lymphocytes contain large granules diffusely distributed in cytoplasm. Giemsa stain, bar = 20 μm.

cats with alimentary form). Particular data on results of the cytological examination are presented in Table 2; microscopic pictures of two cases are presented in Fig. 1 and Fig. 2. Therapy was introduced in 5 of the 6 cats. In two cases palliative therapy with glucocorticoids was conducted, in two cases chemotherapy with COP protocol, and therapy with Masitinib in one case. Particular data on mode of therapy, response, time from diagnosis to death, and cause of death are presented in Table 3. The median of survival time for cats treated with anticancer therapy was 9 months, the median of survival time for cats treated with glucocorticoids was 1.5 months.

Discussion

As was shown in other studies, diagnosis of LGL in cats can be easily obtained by fine-needle biopsy and cytological examination of collected samples. Moreover, commonly the morphology of neoplastic cells, especially the presence of azurophilic cytoplasmic granules is more obvious in cytologic than histologic slides (Franks et al. 1986, Roccabianca et al. 2006, Tsuboi et al. 2010). Because of the above mentioned points, it seems that cytopathology is a sufficient diagnostic method for large granular lymphomas. In all cases recognized in this study typical features of large granular lymphocytes were clearly visible during cytological examination. In unequivocal cases precise diagnosis can be obtained based on immunohistochemical staining using antibodies detecting the CD3 antigen (present only in cytotoxic T lymphocytes but not in natural killer cells) or granzyme B present in normal large granular lymphocytes (Darbes et al. 1997, Endo et al. 1998, Tsuboi et al. 2010, Moore et al. 2012). However, because there is no information about the association between the presence of these markers and biological behavior, methods of treatment and prognosis, immunostaining has no additional benefit in routine practice. In all presented cases samples were of good quality, were characterized by moderate but usually high cellularity, and neoplastic cells were well preserved. There was no doubt as to diagnosis. Mast cell tumors were excluded in every case by negative staining with toluidine blue.

Although LGLs has been recognized in patients of various age, the mean age of onset is 9 years (Kirya et al. 1997, Endo et al. 1998, Roccabianca et al. 2006). In the present study the youngest cat was older than 9 years of age, and the median age was 12 years. In general, the alimentary form of feline lymphomas are recognized in older patients, and the majority of patients in this small group had this anatomic form of lymphoma. The data and results of the present study do not indicate gender or breed predilection for LGL occurrence in cats.

The small intestine is the most common site of origin in cases of LGL in cats, but mesenteric lymph nodes are also commonly involved with neoplastic process (Karyia et al. 1997, Derbes et al. 1998, Endo et al. 1998, Roccabianca et al. 2006, Kirk et al. 2008, Moore et al. 2012). In the present study the alimentary form was recognized in 4 of 6 cats; additionally, in the cat with a multisystemic form, enlargement of abdominal lymph nodes was also detected. All animals were presented at an advanced stage, and therefore the main pathology was marked mesenteric lymphadenomegaly and segmental thickening of intestinal loops. Such presentation is typical for feline LGL and we were able to easily discover an abnormal abdominal mass during abdominal palpation and confirm this by ultrasonography. Because autopsies were not performed in our cases, we do not know what was the primary site of neoplastic growth. However, from a practical point of view this is not important, since chemotherapy is the basic method of therapy. Because the nasal form of LGL in cats is sparsely described in the literature, one case in the present study seems to be unusual. The clinical signs suggesting chronic inflammation of the nasal cavity in this animal were present one year before final diagnosis. Periodical treatment with injection of glucocorticoids allow the problem to be controlled, at least at the beginning of the disease. However, with time this treatment was insufficient, and additional diagnostic tests including computed tomography, fine-needle biopsy and cytopathology led to the final diagnosis of large granular lymphoma. It was not established if these clinical signs related to chronic nasal disease were a consequence of LGL from the beginning or if LGL developed as a consequence of chronic rhinitis. Chronic inflammation seems to be involved in the development of LGL, at least in intestinal lymphoma (Roccabianca et al. 2006).

According to available data, because of the aggressive biologic behavior of the disease in cats, animals are commonly presented in an advanced clinical stage of the disease, with involvement of numerous internal organs and less or more remarkable laboratory abnormalities (Wellman et al. 1992, Roccabianca et al. 2006). Among these the most commonly described are hypoproteinemia, hypocalcemia, hyperbilirubinemia, increased activity of liver enzymes, and elevation of serum creatinine and urea (Wellman et al. 1992, Roccabianca et al. 2006). In the present study regardless of anatomic form, any such serious abnormalities were noted, and some mild deviation could be attributable to advanced age (features indicating mild azotemia were observed only in the

oldest patient). In one cat liver insufficiency developed during treatment with Masitinib. Infiltration of intestinal mucosa with neoplastic lymphocytes can produce protein-losing enteropathy, characterizing by low serum protein concentration (Roccabianca et al. 2006). However, in any case presented here this abnormality was present, probably because of segmental, but not diffuse involvement of the small intestine was observed. The presence of neoplastic cells in peripheral blood is a typical feature of feline large granular lymphomas, sometimes leukocytosis is marked, reaching 70000 white blood cells per µl of blood (Karyia et al. 1997, Darbes et al. 1998, Roccabianca et al. 2006). Surprisingly, leukocytosis was observed only in 2 of 6 cats in this study; however, even in these animals, elevation of leukocyte number was mild and was related to increased granulocyte count, without the presence of neoplastic cells in the blood.

As was mentioned above, cytology is an excellent method of diagnosis in cases of large granular lymphomas (Karyia et al. 1997, Derbes et al. 1998, Roccabianca et al. 2006, Tsuboi et al. 2010). In all presented cases we were able to obtain the final diagnosis easily, and samples were rich in cells that had a distinct morphology. Fine to large cytoplasmic granules were clearly visible in at least 60% of cells, but in the majority of cases cells with cytoplasmic granules comprised nearly all cells with lymphocyte morphology. As has been shown in other studies, regardless of cell origin (cytotoxic T lymphocytes vs. NK cells), distribution and size of cytoplasmic granules varies. In the present study in half of the cases granules were grouped in one area of cytoplasm, and in the other half of cases they were uniformly distributed throughout the entire cytoplasm. The morphology of neoplastic granular lymphocytes can be immature (nuclei with prominent nucleoli) or mature (Roccabianca et al. 2006); in our cases this second type of cell predominated. Unfortunately, no features of cellular morphology, including differentiation of neoplastic cells, permit the prediction of tumour behavior and response to therapy (Roccabianca et al. 2006). This was also true in our patients; response to therapy and time of survival were not related to microscopic features of neoplastic lymphocytes.

In the vast majority of cases response to therapy in cats with LGL is poor and prognosis is usually grave (Kirya et al. 1997, Krick et al. 2008). In one study including 23 cats with LGL, only one complete response and six partial responses were noted in patients which received chemotherapy as their initial treatment. Among 3 cats treated with conventional chemotherapy in this study, in two cases reduction of mass, and stabilization of the disease in the third cat were noted; median survival time for these animals was 9 months. This seems a very good outcome, since the median survival time for cats with LGL treated with chemotherapy is very short, and averages from 19 days to 2 months (Franks et al. 1986, Roccabianca et al. 2006, Krick et al. 2008, Moore et al. 2012). It seems that the cause of such good results of conventional chemotherapy in some cases could be the fact that there was no serious involvement of internal organs, including the liver and kidney (as was shown by biochemistry). There are no prognostic factors in cases of feline LGLs, and the suggestion that cell immaturity or specific immunophenotype can be related to poor prognosis has not been proved (Roccabianca et al. 2006). In our study, LGLs with mature and immature morphology were recognized, but the results of cytopathology, as well as anatomic form were not related to outcome.

Many cats with LGLs are euthanized at the time of diagnosis, because of the advanced stage of the disease, poor general state, and unfavorable diagnosis. Additionally, the alimentary form of feline LGL is commonly accompanied by perforation of the intestinal wall and subsequent peritonitis (Karyia et al. 1997). None of the cats in the present study was euthanized at the time of diagnosis; some of the cats were treated with glucocorticoids as palliative therapy and, despite the advanced stage, and no obvious response to the drug, survival time for these patients was at least 1.5 months. One cat with the systemic form was treated with Masitinib, and although there was no reduction of neoplastic mass size, death of the animal was not related to progression of disease.

In conclusion, large granular lymphomas, especially the alimentary form, are a relatively common type of lymphoma in cats. Simple diagnostic methods such as clinical examination, imaging techniques and routine cytology are sufficient in the majority of cases. Despite aggressive behavior and poor general prognosis, conventional chemotherapy leads to a good response in some treated cats, regardless of anatomic form and histologic grade of neoplastic cells.

References

Aitken-Palmer C, Isaza R, Dunbar M, Blackwood S, Gerlach T, Russell K (**2011**) Anterior uveitis as an atypical presentation of large granular lymphoma in a caracal (*Caracal caracal*). Vet Ophthalmol 14: 337-340.

Chino J, Fujino Y, Kobayashi T, Kariya K, Goto-Koshino Y, Ohno K, Nakayama H, Tsujimoto H (**2013**) Cytomorphological and immunological classification of feline lymphomas: clinicopathological features of 76 cases. J Vet Med Sci 75: 701-707.

Darbes J, Majzoub M, Breuer W, Hermanns W (**1998**) Large granular lymphocyte leukemia/lymphoma in six cats. Vet Pathol 35: 370-379.

Endo Y, Cho KW, Nishigaki K, Momoi Y, Nishimura Y, Mizuno T, Goto Y, Watari T, Tsujimoto H, Hasegawa A (**1998**) Clinicopathological and immunological characteristics of six cats with granular lymphocyte tumors. Comp Immunol Microbiol Infect Dis 21: 27-42.

Franks PT, Harvey JW, Calderwood Mays M, Senior DF, Bowen DJ, Hall BJ (**1986**) Feline large granular lymphoma. Vet Pathol 23: 200-202.

Kariya K, Konno A, Ishida T (**1997**) Perforin-like immunoreactivity in four cases of lymphoma of large granular lymphocytes in the cat. Vet Pathol 34: 156-159.

Krick EL, Little L, Patel R, Shofer FS, Sorenmo K, Clifford CA, Baez JL (**2008**) Description of clinical and pathological findings, treatment and outcome of feline large granular lymphocyte lymphoma (1996-2004). Vet Comp Oncol 6: 102-110.

Moore PF, Rodriguez-Bertos A, Kass PH (**2012**) Feline gastrointestinal lymphoma: mucosal architecture, immunophenotype, and molecular clonality. Vet Pathol 49: 658-668.

Neta M, Naigamwalla D, Bienzle D (**2008**) Perforin expression in feline epitheliotropic cutaneous lymphoma. J Vet Diagn Invest 20: 831-835.

Pohlman LM, Higginbotham ML, Welles EG, Johnson CM (**2009**) Immunophenotypic and histologic classification of 50 cases of feline gastrointestinal lymphoma. Vet Pathol 46: 259-268.

Roccabianca P, Vernau W, Caniatti M, Moore PF (**2006**) Feline large granular lymphocyte (LGL) lymphoma with secondary leukemia: primary intestinal origin with predominance of a CD3/CD8 αα phenotype. Vet Pathol 43: 15-28.

Tsuboi M, Uchida K, Park ES, Kotera Y, Seki T, Takahashi M, Nakayama H (**2010**) Systemic T cell large granular lymphocyte lymphoma with multifocal white matter degeneration in the brain of a Japanese domestic cat. J Vet Med Sci 72: 795-799.

Wellman ML, Hammer AS, DiBartola SP, Carothers MA, Kociba GJ, Rojko JL (**1992**) Lymphoma involving large granular lymphocytes in cats: 11 cases (1982-1991). J Am Vet Med Assoc 201: 1265-1269.