Cytomorphological and Immunological Classification of Feline Lymphomas: Clinicopathological Features of 76 Cases

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ABSTRACT. Of the various classification systems for non-Hodgkin’s lymphoma, the updated Kiel classification is valuable for veterinary practice, because of its utility to classify the subtypes not only by histopathology but also by cytomorphology. However, there are only a few reports of small number of feline lymphomas to apply the updated Kiel classification. In this study, immunological subtypes and morphology of 76 feline lymphomas were evaluated and classified into subtypes of the updated Kiel classification. Of the cases, 49% were T-cell lymphoma, 25% were B-cell lymphoma and 26% were undetermined immunological subtype. Based on the updated Kiel classification, most subtypes were identified also in feline lymphomas as in dogs and humans. Globule leukocyte lymphoma was specific for cats and relatively common in feline alimentary lymphomas. Of the present cases, 64% were high-grade subtypes, whereas 36% were low-grade subtypes. The present study indicated that feline lymphomas could be morphologically classified by the modified updated Kiel classification.

KEYWORDS: feline, lymphoma, updated Kiel classification.


Lymphomas are common malignant neoplasms in cats. Recently, there are increased interest about evaluation of prognosis and choice of treatment. Also, lymphomas are known to have a great diversity of cytological morphology and biological behavior.

Various classification systems for non-Hodgkin’s lymphomas in humans have been established so far [24]. The updated Kiel classification (1988), revised from the Kiel classification (1974), has been used mainly in Europe. At the same period, the WF (Working formulation) classification has been proposed by National Cancer Institute (1982) and used mainly in North America. Nowadays, updated WHO (World Health Organization) classification (2001) is becoming a mainstream after several stages, such as the updated Kiel classification and the REAL (Revised European-American classification of Lymphoid neoplasms) classification (1994) in humans. Although most of classifications require histopathology, it is not always possible to perform tissue biopsy at the time of diagnosis in veterinary medicine. Of those, the updated Kiel classification [21] is thought to be most convenient in veterinary clinical oncology, because it is utilizable to determine subtypes by cytopathological morphology, nor always requires histopathological observation.

In veterinary clinical science, some studies indicate that the updated Kiel classification is applicable to canine lymphomas [6, 7, 17, 19, 22]. One of the studies reported that the first remission duration and the overall survival time were significantly different among the subtypes of lymphomas [17]. It was also reported in another canine research that both intra- and inter-observer reproducibilities were good in the updated Kiel classification [22].

However, there have been only a few reports that the updated Kiel classification has been applied in feline lymphomas. Immunoblastic and lymphoblastic subtypes were found in the study of cats with spinal lymphoma [20]. Centroblastic, immunoblastic and lymphocytic subtypes were found in the study of feline immunodeficiency virus (FIV)-infected cats with lymphoma [1]. However, each report included only 8 classified cases, and they were far from discussing the entirety of feline lymphomas.

The aim of this study was to classify a large number of cats with lymphoma by the updated Kiel classification and to indicate the clinicopathological features of the subtypes.

MATERIALS AND METHODS

Cases: Seventy-six cats admitted to Veterinary Medical Center of the University of Tokyo and cooperating private veterinary hospitals were diagnosed as lymphoma by cytopathological and +/- histopathological examination between 2001 and 2006 and included in the study. From the cases, samples for the cytology and/or histopathology, and for the immunological analysis were obtained. Cases with suspected
lymphoma that insufficient samples to diagnose and classify were obtained were excluded in the study.

**Determining the immunological subtypes:** To determine the immunological subtype of lymphomas, two procedures were employed. One procedure was to analyze the genetic status of TCRγ (T-cell receptor gamma chain) and IgH (Immunoglobulin heavy chain) by PCR. DNA samples of all cases were extracted from fresh lymphoma cells obtained by fine needle aspiration or biopsy, or from formalin-fixed paraffin-embedded tissues of biopsy specimens. The primers and protocols for PCR analyses were used as previously described [15, 18, 26]. When a monoclonality or an oligoclonality was detected with the hetero-duplex analysis, the lymphoma cells were decided to have the clonal nature of the lymphoma as well as immunological nature of the clonal cell lineage.

Immunohistochemistry was chosen as another way to determine the tumor phenotype [18]. Twenty-eight formalin-fixed paraffin-embedded lymphoma tissues were sliced into 4 µm sections and prepared on slides coated with 3-aminopropyl-triethoxy-silane. The slides were dehydrated through graded alcohols and treated in 1% citrate buffer solution (pH6.0) under the autoclave at 120°C for 15 min. Then, endogenous peroxidase activity was eliminated by 1% hydrogen peroxide in methanol, and blocking was processed by 8% skim milk in Tris-buffered saline. The sections were incubated with the panel of primary antibodies specific for CD3 (T-cell marker, dilution 1:300, A0452, Dako, Carpenteria, CA, U.S.A.) and CD79a (B-cell marker, dilution 1:50, M7051, Dako) overnight at 4°C. Negative and positive control sections were also incubated at this stage. After primary incubation, they were treated by biotin-conjugated anti-mouse IgG antibody or anti-rabbit IgG antibody (Both from Cappel, Aurora, OH, U.S.A.) and by horse-raddish peroxidase labeled streptavidin (Dako). Finally, the immunolabeling was visualized by 0.05% 3,3'diaminobenzidine tetrahydrochloride with 0.01% hydrogen peroxide. Although the primary antibodies were for humans, they were validated to use for cats in previous studies about feline lymphoma [3, 4, 18, 25]. If results of the immunological status between genetic analyses by PCR and immunohistochemistry were discordant, the result of immunohistochemistry was priori-

**Morphological evaluation:** Wright-Giemsa stained cytological specimens of all cases and Hematoxylin-Eosin stained histopathological specimens of 28 cases were reviewed to determine morphological characteristics by two veterinary clinical pathologists (Chino, J. and Fujino, Y.). By evaluating both immunological and cytological status, the subtype of the lymphoma was assigned according to the updated Kiel classification [21]. In cases with neoplasms composed of large granular cells, toluidine blue and naph-thol AS-D chloroacetate esterase stainings were performed in addition to immunopathological and genetic analyses to distinguish between mastocytoma and lymphoma. If there was a case unmatched to any subtype of the updated Kiel classification [21], a new subtype was proposed (Table 2).

**Clinicopathological characteristics:** Medical records of the cases were reviewed to study clinicopathological characteristics. Age, sex, the WHO clinical stage, anatomical form, swelling of superficial lymph nodes, splenomegaly, hepatomegaly, presence of a mediastinal mass, packed corpuscular volume (PCV), hypercalcemia related to lymphoma and FIV or feline leukemia virus (FeLV) infection at the time of diagnosis were reviewed. All cases were clinically staged with the complete blood cell count profile including observation of the buffy coat smear, and abdominal and thoracic radiography and ultrasonic echography.

**RESULTS**

**Immunological origin and morphological grades:** Both the PCR analysis and immunohistochemistry were performed in 28 lymphoma cases (Table 1). Compared to the results with immunohistochemistry, the sensitivity of the primer for IgH was 89% (8/9), and the specificity of that was 74% (14/19). The sensitivity of the primer for TCRγ was 29% (5/17), and the specificity of that was 100% (11/11).

Of the 76 cases with lymphoma, 37 cases (48.7%) were determined as T-cell origin and 19 cases (25.0%) as B-cell origin. Twenty cases (26.3%) remained undetermined, because the immunological origin of them was undetectable by either the PCR analysis or immunohistochemistry. As the grades were divided according to the morphology of the updated Kiel classification, 27 cases (35.5%) and 49 cases (64.5%) were classified into low-grade and high-grade subtypes, respectively (Table 2). The reproducibility of morphological classification of feline lymphomas was excellent (100% concordance) between the two veterinary clinicopathologists in the present study. Also, the morphological classification evaluated between cytopathological and histopathological specimens was agreed by the two veterinary clinicopathologists.

The numbers of low-grade and high-grade cases in T-cell lymphoma were nearly equal (18 and 19, respectively). However, there was a dominant tendency to high-grade cases (17 cases) compared with low-grade cases (2 cases) in B-cell lymphoma. On the other hand, the case numbers of T-cell and B-cell lymphoma were almost the same in high-grade cases (19 and 17, respectively), whereas T-cell lymphoma (18 cases) was much more frequently observed than B-cell lymphoma (2 cases) in low-grade cases.

**Anatomical forms and morphological grades/immunological origin:** The number of cases and ratio of each anatomical form of lymphoma were tabulated (Table 3). Nasal and orbital lymphomas were assembled together, because lesions

| Table 1. Results of immunophenotype and clonal gene rearrangement in 28 cases examined by both analyses |
|-----------------------------------------|--------------|--------------|--------------|
| Clonal gene rearrangement               | CD3 (+), CD79a (+) | CD3 (+), CD79a (–) | CD3 (–), CD79a (–) |
| IgH (+), TCRγ (–)                       | 4            | 8            | 1            |
| IgH (–), TCRγ (+)                       | 5            | 0            | 0            |
| IgH (–), TCRγ (–)                       | 8            | 1            | 1            |
in most cases were already spread in both regions and it
could be difficult to determine where the primary focus was.

In the low-grade lymphomas, alimentary, renal, mediastinal,
multicentric and hepatic forms were seen. Alimentary
form cases were most frequently found and accounted for
81.5% of the low-grade cases. Among the high-grade lym-
phomas, alimentary form was also the most common type
(30.6%), and there were several other forms in which nasal/orbital (20.4%),
mediastinal (16.3%), multicentric (16.3%) and cutaneous (8.2%) forms had several numbers of cases.
The patients of the mediastinal form and the multicentric
form were more in the high-grade than those in the low-
grade. The patients of nasal/orbital or cutaneous form were
seen only in the high-grade.

The relationship between anatomical forms and tumor cell
phenotypes was also tabulated (Table 3). In the alimentary
form, T-cell lymphoma showed a high proportion (59.5%)
compared to B-cell lymphoma (18.9%). In the mediastinal
form, five of nine cases (55.6%) were T-cell lymphoma,
whereas three cases (33.3%) were B-cell lymphoma. All
of nasal/orbital lymphomas with detectable immunological
origin were the B-cell subtype. All of four cutaneous lym-
phomas were T-cell origin. Of three renal lymphomas, two
cases were T-cell origin, and one case was undetermined.

Morphological subtypes based on the updated Kiel clas-
sification: By the microscopic observation of the cytological
specimens of lymphomas (Fig. 1), morphological features of
the cells from most of the cases could be classified according
to the updated Kiel classification (Table 2).

In T-cell subtypes, lymphocytic, prolymphocytic, pleo-
morphic small cell, lymphoblastic, immunoblastic, pleomor-
phic medium/large cell and anaplastic large cell types were
identified (Tables 2 and 4). Two subtypes were proposed,
because there were cases that matched none of the estab-
ishased subtypes. One was the globule leukocyte type that
was undefined in the updated Kiel classification, since it was
unobserved in human lymphomas. The globule leukocyte
neoplasm is a kind of large granular lymphoma [5, 11, 12,
14], which is distinct from other lymphomas by the feature
of its unique granules in the cytoplasm (Fig. 1h). The other
subtype was the T-centroblastoid type, of which immuno-
logical subtype predicted from cell morphology was B-cell
origin but the detected origin came out to be T-cell.

In B-cell subtypes (Tables 2 and 5), there were only
two low-grade cases belonging to the lymphocytic and
prolymphocytic types. In the high-grade, lymphoblastic, im-
umonoblastic, centroblastic, Burkitt-like and anaplastic large
cell types were observed. There was also a proposed B-cell

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<th>Table 2. Cytopathologically classified 56 feline lymphomas based on the updated Kiel classification</th>
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<td><strong>Low-grade</strong></td>
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<td>---------------------------------------------------------------</td>
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<tr>
<td>Lymphocytic</td>
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<td>Prolymphocytic</td>
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<td>Lymphoplasmacytic</td>
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<td>Centrocytic</td>
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| **High-grade** | **T-cell lymphoma** | **Number of cases** |
|---------------------------------------------------------------|
| G杼ule leukocyte* | 8 |

*Subtypes of feline lymphoma without human counterparts.

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<tr>
<th>Table 3. Anatomical forms and morphological grades/immunological origin of 76 feline lymphomas</th>
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<td><strong>Low-grade</strong></td>
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<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Alimentary</td>
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<tr>
<td>Nasal/Orbital</td>
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<tr>
<td>Mediastinal</td>
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<td>Multicentric</td>
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<td>Cutaneous</td>
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<td>Renal</td>
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<td>Hepatic</td>
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<td>Brain</td>
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<tr>
<td>Skeletal muscle</td>
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<td>Total (%)</td>
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The numerals represent the number of cases.
Fig. 1. Morphology of feline lymphomas. Wright-Giemsa stained. (a) Lymphocytic type (low-grade) composed of small cells with scant cytoplasm and a round nucleus which has clumped chromatin and no nucleolus. Magnification × 600. (b) Lymphoblastic type (high-grade) composed of medium to large round cells with a round nucleus which has uncondensed chromatin and obscure nucleoli. Magnification × 600. (c) Immunoblastic type (high-grade) composed of medium to large round cells with a round nucleus which has fine chromatin and a prominent central nucleolus. Magnification × 600. (d) Centroblastic type (high-grade) composed of medium to large round cells with basophilic cytoplasm and an eccentric nucleus which has fine chromatin and several eccentric nucleoli. Magnification × 600. (e) Burkitt-like type (high-grade) composed of small to medium cells with vacuolated basophilic cytoplasm and round nucleus which has fine chromatin and minute nucleoli. Magnification × 600. (f) Pleomorphic medium and large cell type (high-grade) composed of medium to large cells with an irregular shaped and cleaved nucleus. Magnification × 600. (g) Anaplastic large cell type (high-grade) composed of large to extremely large cells with polymorphic nuclei. Magnification × 800. (h) Globule leukocyte type (low-grade) composed of medium to large cells with clumped or uncondensed chromatin and granulated cytoplasm. The granules are coarse and surrounded by halos. Magnification × 1,000.
subtype of pleomorphic medium/large cell type which was predicted to be a T-cell subtype assumed from morphological findings.

Clinicopathological characteristics: Clinicopathological information was collected from medical records and reviewed (Tables 4 and 5). Noticeably, four out of five T-lymphoblastic lymphomas presented mediastinal masses. It was also noted that there were no young cats with low-grade lymphoma, while there were juvenile patients with high-grade lymphoma.

Of the 62 cats with lymphoma examined for FeLV and FIV infections at the time of diagnosis, 10 cases (16.1%) were positive for serum FIV antibodies, and 9 cases (14.5%) were positive for FeLV antigens. Two cats had co-infection, and either FeLV- or FIV-infected cases were 17 cats (27.4%) in total. There was no tendency in immunological origin of lymphoma in the 9 FeLV-infected cats (B-cell, 4 cases; T-cell, 3 cases; Undetermined, 2 cases), whereas 6 out of 7 FIV-infected cases with detectable immunological origin were of B-cell lymphoma. It was also noteworthy that all lymphomas in cats infected with FeLV and/or FIV showed high-grade morphology.

DISCUSSION

In the present study, the number of cats with high-grade lymphoma was larger than that with low-grade lymphoma. It was the similar observation as in canine lymphomas [6, 17, 19]. However, the proportional tendency of subtypes in feline lymphomas was different from that in canine lymphomas. Centroblastic lymphoma was the most common type in canine lymphomas [6, 17, 19], whereas there were few predilections of subtypes in feline lymphomas. In addition to the subtypes, the proportional tendency of anatomical forms in feline lymphomas was also different from that in canine lymphomas. Multicentric lymphoma was the most common form in canine lymphomas [6, 17, 19], whereas alimentary lymphoma was the most in feline lymphomas (Table 3). Thus, pathogenesis and biological behavior can be different among animal species.

Low-grade lymphomas showed high prevalence of alimentary form and T-cell subtypes. One of the reasons was...
considered to be the influence of lymphocytic/prolymphocytic and globule leukocyte lymphomas, each of which was nearly half of the T-cell low-grade cases in the present study. The globule leukocyte lymphoma can be classified as low-grade lymphomas, since it is a kind of large granular lymphocyte lymphomas composed of neoplastic mature lymphoid cells, and well-differentiated lymphomas belong to low-grade in the updated Kiel classification [21, 24]. The globule leukocyte is assumed to be derived from a cytotoxic T-cell or a natural killer (NK) cell because of its perforin-like reactivity [12] and exists in lymphoid organ of alimentary tract which can be a primary locus of tumorigenesis [5, 11, 12, 14].

To determine the lymphoid lineage, 28 lymphoma cases could be examined with both immunohistochemistry and genetic clonality analysis of antigen receptor rearrangement by PCR (Table 1). Comparison between the two analyses showed similar results with a previous study [18]. Of 17 cases with T-cell lymphoma determined by immunohistochemistry, 4 cases (24%) showed clonal rearrangement of IgH. Such a repugnant phenomenon in feline lymphoma has been already reported and discussed previously as the cross-lineage rearrangement [18].

In the present study, 20 of 76 cases showed undetermined lineage of lymphoma. It was thought to be caused by the limitations of reactivity of immunohistochemistry and PCR analyses, although a few cases might indicate non-B- and non-T-cell lymphomas. Immunohistochemistry using the CD79a antibody has been already reported to be insufficient to determine feline B-cell lymphomas [9, 18]. Primers used with PCR for antigen receptor rearrangement have been limited to determine the cellular lineage of feline lymphomas [15, 18, 26]. Thus, additional antibodies and primers would be required for the analysis of feline lymphomas.

The case number of T-cell lymphoma was observed approximately twice as much as that of B-cell lymphoma in the present study. This was a converse result to a previous study in Australia [9], and it might be due to variation depending on the geographical distribution. Also, a high incidence of alimentary lymphoma and high prevalence of T-cell lymphoma in the alimentary form led to the present result.

The relationship between anatomical forms and immunological subtypes of lymphomas is often discussed in the previous studies. In alimentary lymphoma, while the ratio of T- and B-cell phenotypes varied in previous studies [16, 25, 27], a high incidence of T-cell subtype was observed in the present study. In mediastinal lymphoma, the B-cell subtype was found up to 1/3 of mediastinal cases in the present study, although there was a report about significant correlation with the T-cell phenotype [9]. Meanwhile, most of the nasal lymphomas were reported to be the B-cell subtype [4, 13], and similar prevalence was observed here. Likewise, cutaneous lymphoma was reported to have high frequency of the T-cell phenotype [3], and it was also confirmed in the present study since all of the cutaneous cases showed the T-cell subtype.

By the updated Kiel classification, most cases of feline lymphomas could be classified into the original subtypes observed in human non-Hodgkin’s lymphomas. Although the centroblastic type was the most frequently observed subtype in canine lymphomas [6, 17, 19], it was inconsistent with feline lymphomas. T-lymphoblastic lymphomas were likely to involve the mediastinal mass that resembled canine cases in which significant relationship between lymphoblasts and the mediastinal mass was reported [2].

It also appeared in the present study that all of lymphomas in cats with retrovirus (FIV or FeLV) infection showed high-grade morphology. It is considered that the retrovirus may have a potential in tumorigenesis at the early phase of the lymphoid differentiation. Especially, FeLV is susceptible to infect dividing cells [8]. Lymphomas in cats with FIV infection had high prevalence of the B-cell subtype in the present study, and it was similar to previous studies of FIV-associated lymphoma [1, 10]. Although the mechanism is unclear, B-cell stimulation in FIV-infected cats may have influenced on B-cell lymphomagenesis.

There were several cases that the immunological subtype suspected by morphology was inconsistent with the actual immunological origin, such as B-cell lymphoma of the pleomorphic medium and large cell type and T-cell lymphoma of the centroblastoid type. Since similar phenomenon is reported in canine lymphomas [6, 7, 23], it was shown that morphology cannot always indicate the aspects of tumor cells in feline lymphomas either.

Consequently, the updated Kiel classification can be basically applied to the cases with feline lymphoma. Lymphoid neoplasms have considerable variations in cellular origin and biological behavior. It is important to classify closely into subtypes to extend scientific knowledge and to have better understanding of biological behavior of lymphomas. Classifying feline lymphomas will be useful to predict the prognosis of the cases and to develop appropriate therapeutic protocols in each subtype of lymphomas.

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